Qualitative Modeling and Simulation of Gene Regulatory Networks

Hidde de Jong

INRIA Grenoble - Rhône-Alpes

Hidde.de-Jong@inria.fr http://ibis.inrialpes.fr

UNIVERSITE JOSEPH FOURIER
SCIENCES TECHNOLOGIE MEDECINE

INSTITUT NATIONAL DE RECHERCHE EN INFORMATIQUE ET EN AUTOMATIQUE
INRIA
centre de recherche GRENOBLE - RHÔNE-ALPES

INRIA Grenoble - Rhône-Alpes and IBIS

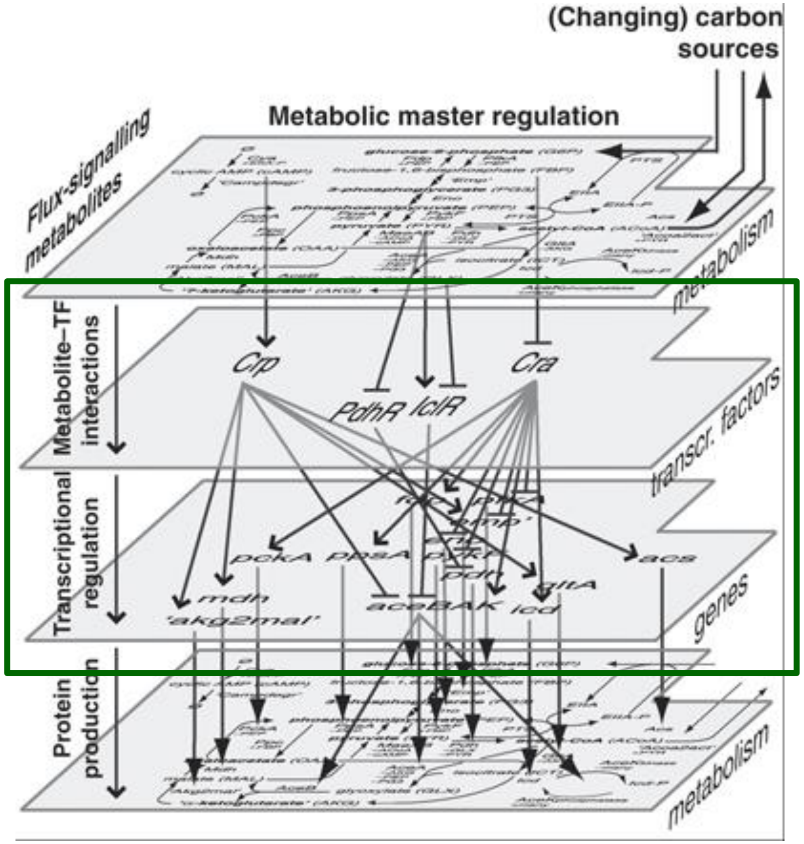
- IBIS: systems biology group of INRIA and Université Joseph Fourier/CNRS
  - Analysis of bacterial regulatory networks by means of models and experiments
  - Biologists, computer scientists, mathematicians, physicists, …

Overview

1. Gene regulatory networks in bacteria
2. Novel methods for measuring gene expression
3. Quantitative modeling of gene regulatory networks
    - Ordinary differential equations
    - Stochastic master equations
4. **Qualitative modeling of gene regulatory networks**
    - **Piecewise-linear differential equations**
5. **Conclusions and perspectives**

Gene regulatory networks

- **Gene regulatory networks** control changes in gene expression levels in response to environmental perturbations

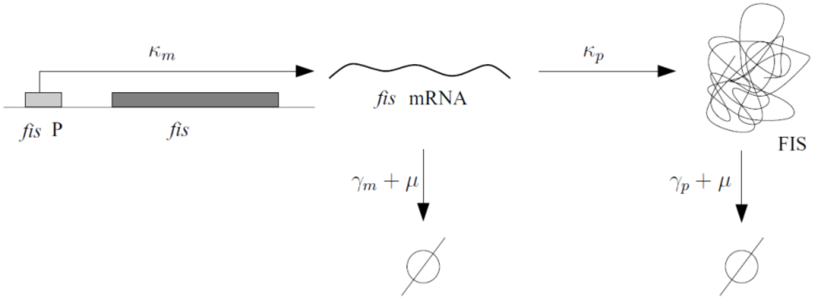


- Gene regulatory networks consist of genes, gene products, signalling metabolites, and their mutual regulatory interactions

  Global regulators of transcription involved in glucose-acetate diauxie in *E. coli*

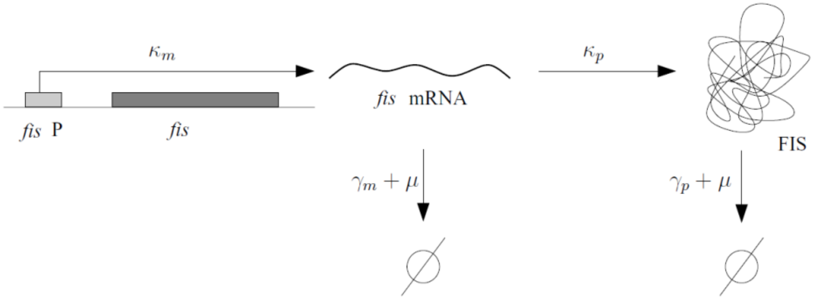
Kotte *et al*. (2010), *Mol. Syst. Biol*., 6: 355

Gene expression



- Typically, and simplifying quite a bit, **gene expression** in bacteria involves:
  - Transcription by RNAP (mRNA)
  - Translation by ribosomes (proteins)
  - Degradation of mRNA and protein

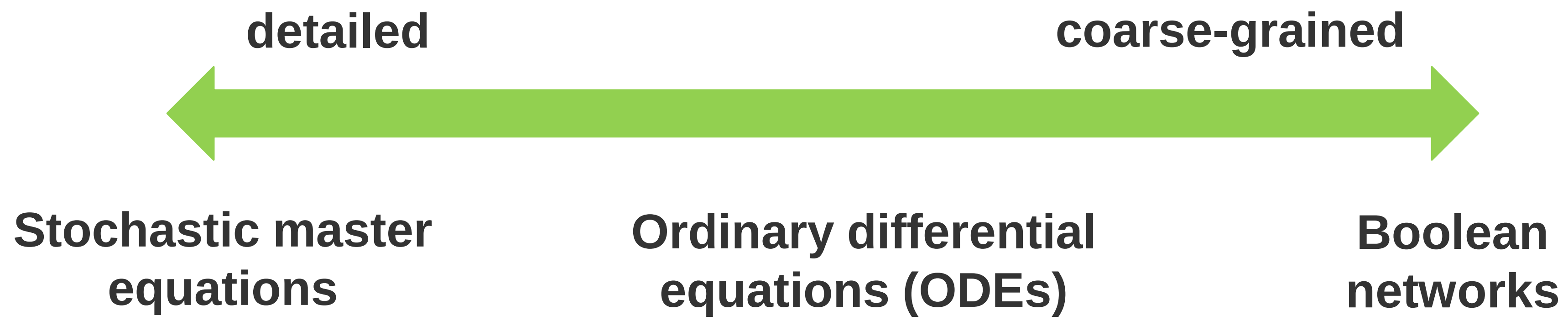
Regulation of gene expression



- Typically, and simplifying quite a bit, **regulation of gene expression** in bacteria involves:
  - Transcription regulation by transcription factors
  - Translational regulation by small RNAs
  - Regulation of degradation by proteases

Modeling of gene regulatory networks

- Different modeling formalisms exist, describing gene expression on different levels of detail



detailed

coarse-grained

Stochastic master equations

Ordinary differential equations (ODEs)

Boolean networks

Smolen *et al.* (2000), *Bull. Math. Biol.*, 62(2):247-292

Hasty *et al.* (2001), *Nat. Rev. Genet.*, 2(4):268-279

de Jong (2002), *J. Comput. Biol.*, 9(1): 69-105

Szallassi et al. (2006), *System Modeling in Cellular Biology*, MIT Press

Bolouri (2008), *Computational Modeling of Gene Regulatory Networks*, Imperial College Press

Karleback and Shamir (2008), *Nat. Rev. Mol. Cell Biol.*, 9(10):770-80

Modeling of gene regulatory networks

- Well-established theory for modeling of genetic regulatory networks using ordinary differential equation (ODE) models

Bolouri (2008), *Computational Modeling of Gene Regulatory Networks*, Imperial College Press

Polynikis *et al.* (2009), *J. Theor. Biol.*, 261(4):511-30

- Practical problems encountered by modelers:
  - Knowledge on molecular mechanisms rare
  - Quantitative information on kinetic parameters and molecular concentrations absent
  - Large models
- Even in the case of well-studied *E. coli* network!

Qualitative modeling and simulation

- Possible strategies to overcome problems
  - Parameter estimation from experimental data
  - Parameter sensitivity analysis
  - Model simplifications
- Intuition: essential properties of network dynamics **robust** against reasonable model simplifications
- **Qualitative** modeling and simulation of large and complex genetic regulatory networks using **simplified** models

  de Jong, Gouzé *et al.* (2004), *Bull. Math. Biol.*, 66(2):301-40
- Relation with discrete, logical models of gene regulation

  Thomas and d'Ari (1990), *Biological Feedback,* CRC Press

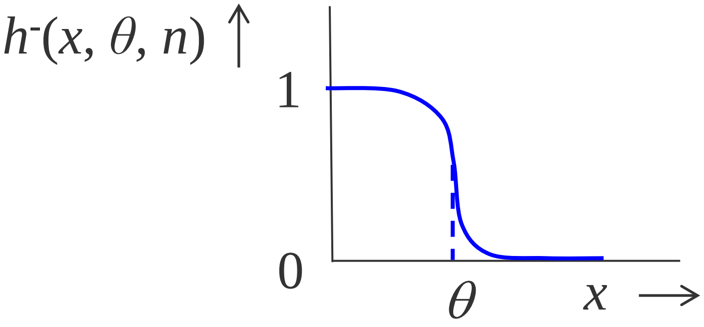
  Kauffman (1993), *The Origins of Order,* Oxford University Press

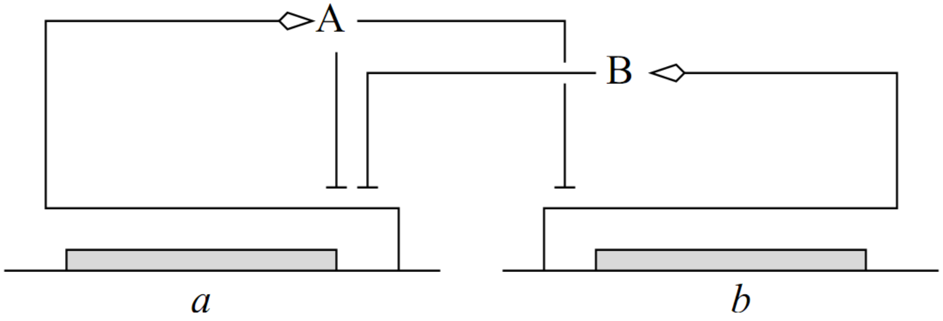
Ordinary differential equation models

- Genetic regulatory networks modeled by ODE models using **sigmoid functions** to describe regulatory interactions

$$\dot{x}_a = \kappa_a \, h^-(x_a, \theta_{a2}, n) \, h^-(x_b, \theta_b, n) - \gamma_a x_a$$

$$\dot{x}_b = \kappa_b \, h^-(x_a, \theta_{a1}, n) - \gamma_b x_b$$





x : protein concentration
θ : threshold concentration
κ, γ : rate constants
n : steepness parameter

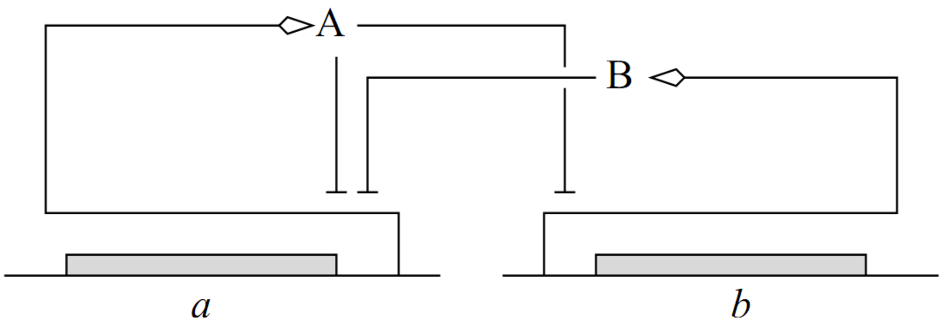
- Expressions of sigmoid functions account for **combinatorial control** of gene expression (AND, OR, NOR, …)

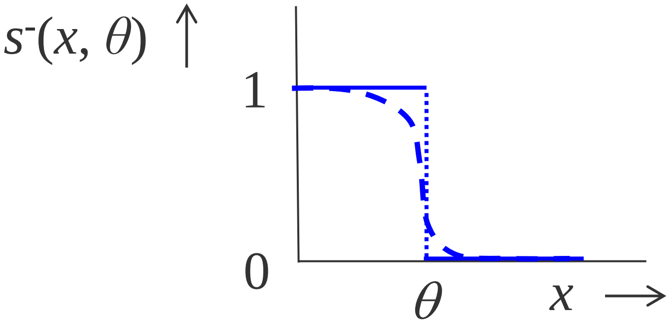
PL differential equation models

- ODE models approximated by means of **step functions** to describe regulatory interactions

$$\dot{x}_a = \kappa_a\, s^-(x_a\,,\ \theta_{a2})\, s^-(x_b\,,\ \theta_b\,) - \gamma_a\, x_a$$

$$\dot{x}_b = \kappa_b\, s^-(x_a\,,\ \theta_{a1}) - \gamma_b\, x_b$$





x : protein concentration

θ : threshold concentration

κ , γ : rate constants

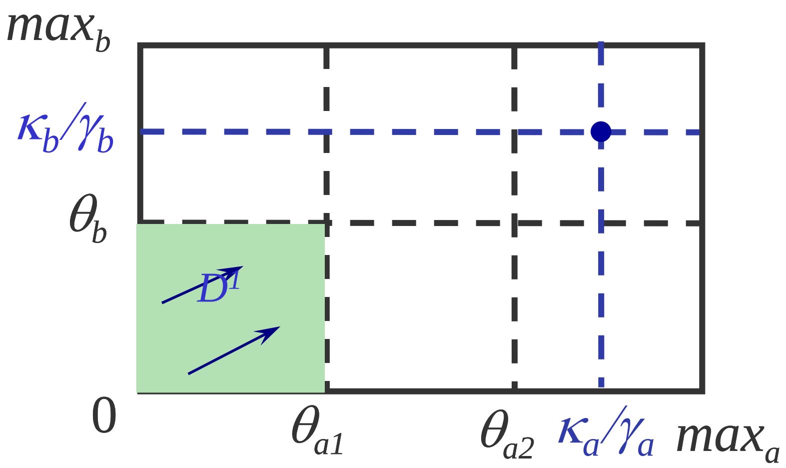
- **Piecewise-linear (PL)** DE models of genetic regulatory networks

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

  Monotone convergence towards **focal point** in regions separated by thresholds



$$\dot{x}_a = \kappa_a - \gamma_a\, x_a$$

$$\dot{x}_b = \kappa_b - \gamma_b\, x_b$$

$$\dot{x}_a = \kappa_a\, s^-(x_a\,,\ \theta_{a2})\ s^-(x_b\,,\ \theta_b\,) - \gamma_a\, x_a$$

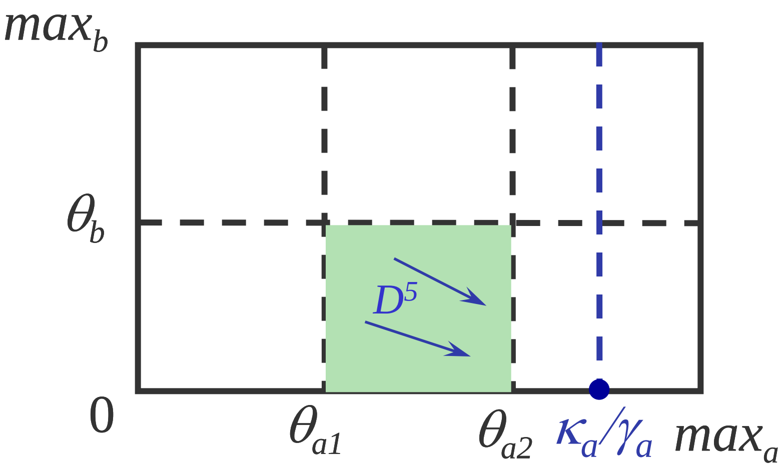
$$\dot{x}_b = \kappa_b\, s^-(x_a\,,\ \theta_{a1}) - \gamma_b\, x_b$$

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

  Monotone convergence towards **focal point** in regions separated by thresholds



$$\dot{x}_a = \kappa_a - \gamma_a\, x_a$$
$$\dot{x}_b = -\,\gamma_b\, x_b$$

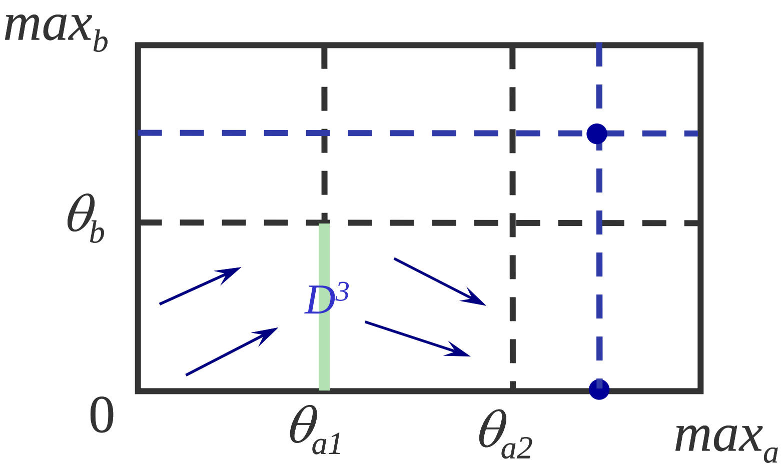
$$\dot{x}_a = \kappa_a\, s^-(x_a\,,\, \theta_{a2})\, s^-(x_b\,,\, \theta_b\,) - \gamma_a\, x_a$$
$$\dot{x}_b = \kappa_b\, s^-(x_a\,,\, \theta_{a1}) - \gamma_b\, x_b$$

Glass and Kauffman (1973), *J. Theor. Biol.*, 39(1):103-29

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

  Instantaneous crossing of regions located on thresholds, or …



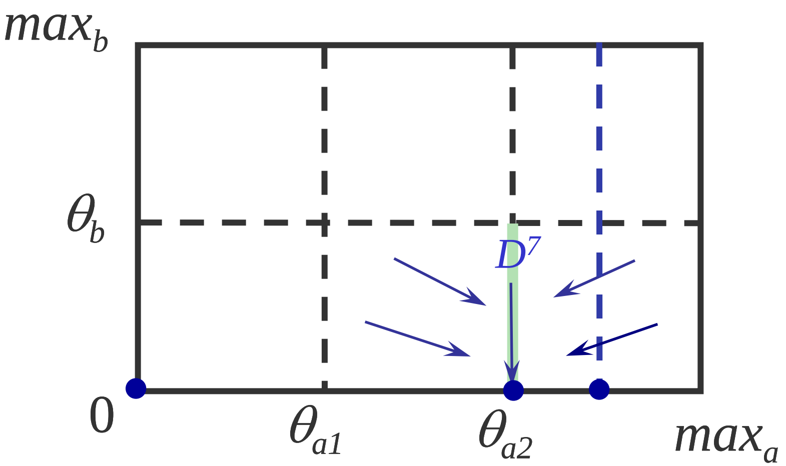
$$\dot{x}_a = \kappa_a\, s^-(x_a\,,\ \theta_{a2})\, s^-(x_b\,,\ \theta_b\,) - \gamma_a\, x_a$$

$$\dot{x}_b = \kappa_b\, s^-(x_a\,,\ \theta_{a1}) - \gamma_b\, x_b$$

Mathematical analysis of PL models

- Analysis of local dynamics of PL models

  … quasi-monotone convergence towards **focal sets** located on threshold hyperplanes



$$\dot{x}_a = \kappa_a\, s^-(x_a\,,\ \theta_{a2})\ s^-(x_b\,,\ \theta_b\,) - \gamma_a\, x_a$$

$$\dot{x}_b = \kappa_b\, s^-(x_a\,,\ \theta_{a1}) - \gamma_b\, x_b$$

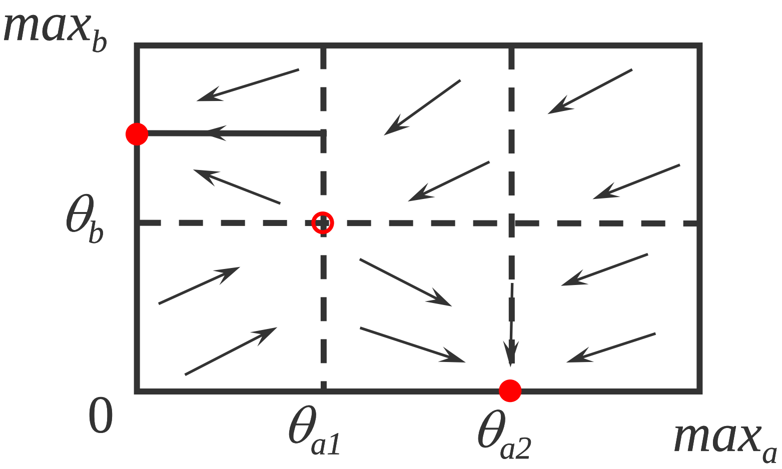
- Extension of PL differential **equations** to differential **inclusions** using Filippov approach

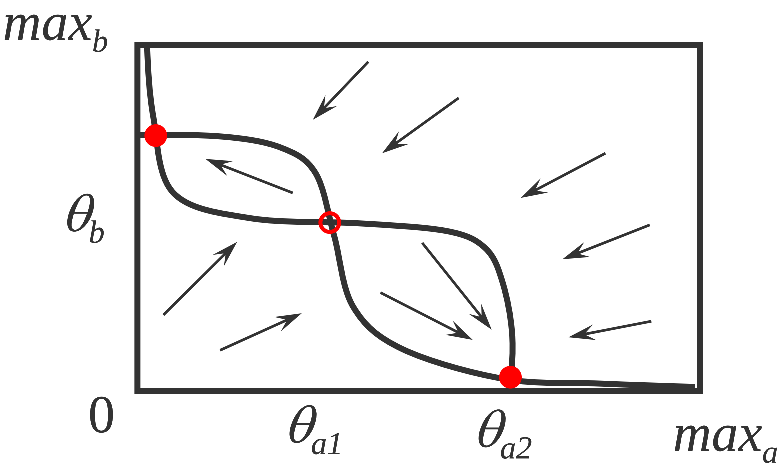
Gouzé and Sari (2002), *Dyn. Syst.*, 17(4):299-316

Qualitative analysis of PL models

- Analysis of global dynamics obtained by piecing together local dynamics in regions

  PL approximation preserves bistability of cross-inhibition network

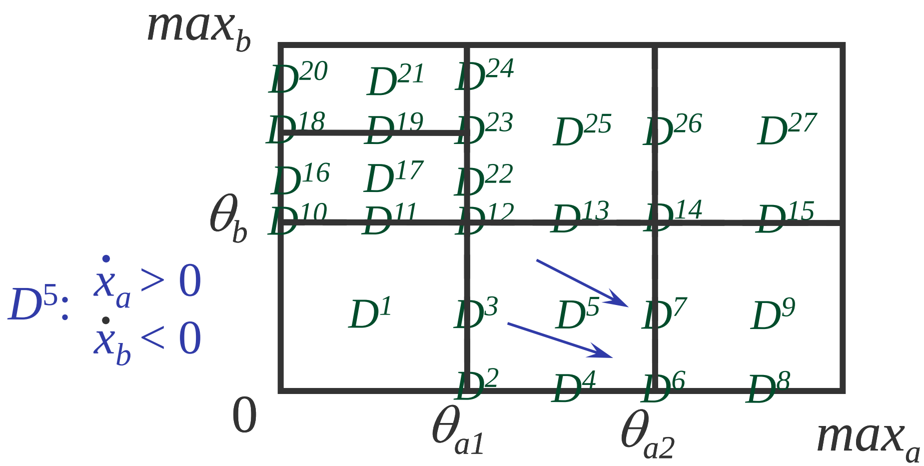




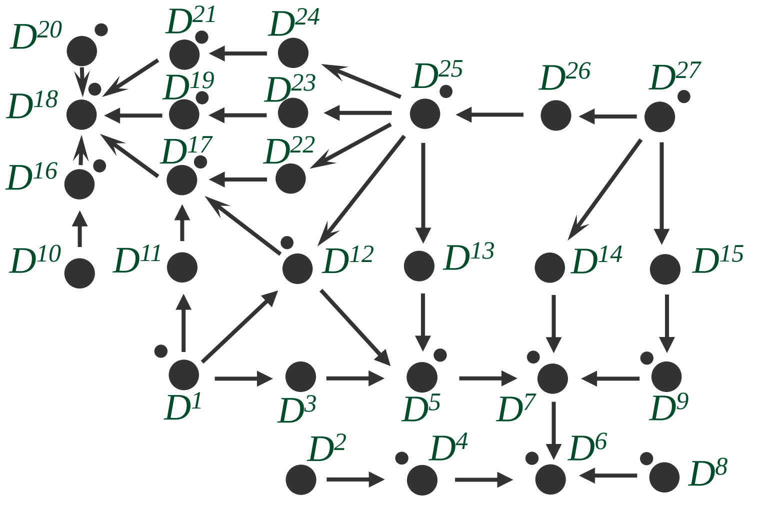
Qualitative analysis of PL models

- State space can be partitioned into regions with **unique** derivative sign pattern
- **Qualitative abstraction** yields state transition graph that provides discrete picture of continuous dynamics

Alur *et al.* (2000), *Proc. IEEE*, 88(7):971-84



D^5: $\dot{x}_a > 0$
$\dot{x}_b < 0$



D^1: $\dot{x}_a > 0$
$\dot{x}_b > 0$

D^5: $\dot{x}_a > 0$
$\dot{x}_b < 0$

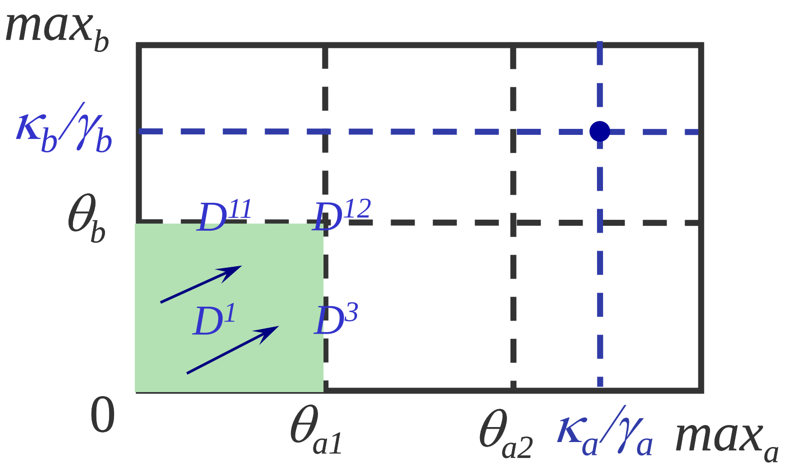
D^7: $\dot{x}_a = 0$
$\dot{x}_b < 0$

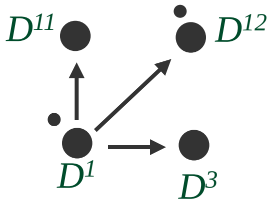
de Jong *et al.* (2004), *Bull. Math. Biol.*, 66(2):301-40

Batt *et al.* (2008), *Automatica*, 44(4):982-9

Qualitative analysis of PL models

- State transition graph gives **conservative approximation** of continuous dynamics
  - Every solution of PL model corresponds to path in state transition graph
  - Converse is not necessarily true!
- State transition graph is **invariant** for given inequality constraints on parameters





$$0 < \theta_{a1} < \theta_{a2} < \kappa_a/\gamma_a < max_a$$

$$0 < \theta_b < \kappa_b/\gamma_b < max_b$$

Batt *et al.* (2008), *Automatica*, 44(4):982-9

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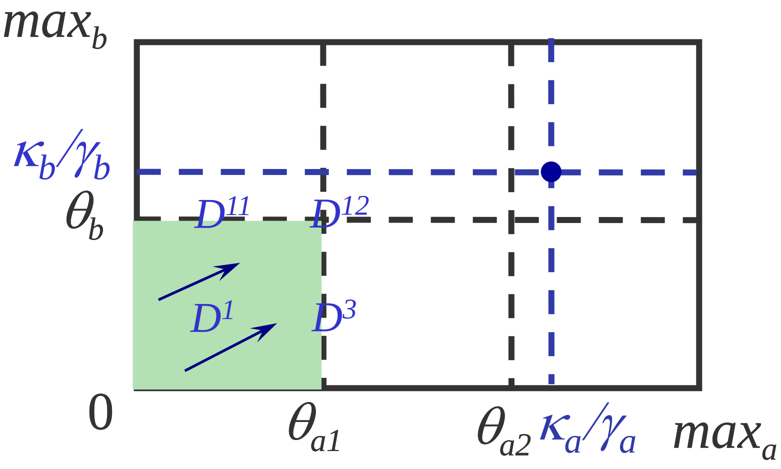

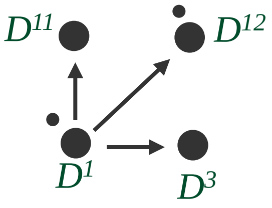

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Batt *et al.* (2008), *Automatica*, 44(4):982-9

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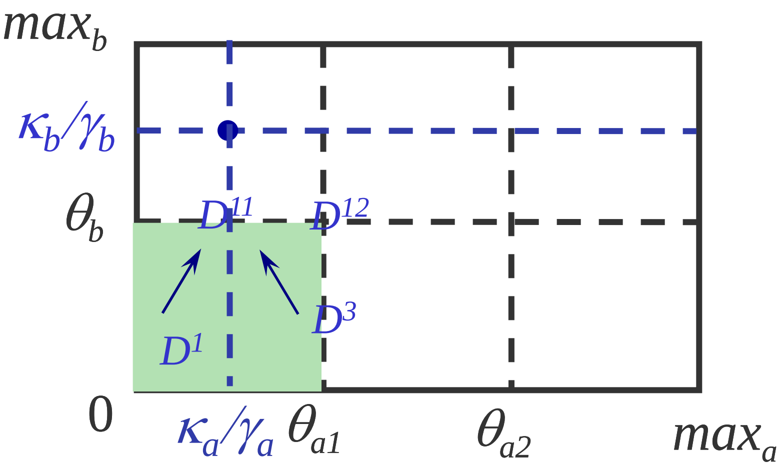
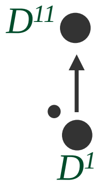




$$0 < \kappa_a/\gamma_a < \theta_{a1} < \theta_{a2} < max_a$$

$$0 < \theta_b < \kappa_b/\gamma_b < max_b$$

Batt *et al.* (2008), *Automatica*, 44(4):982-9

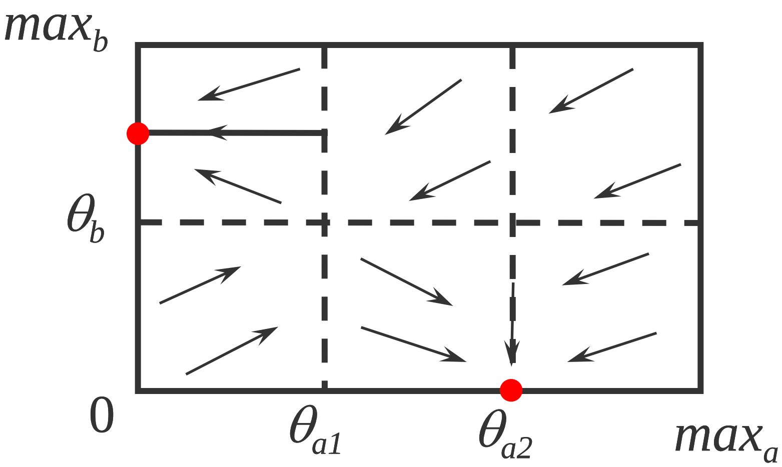
Use of state transition graph

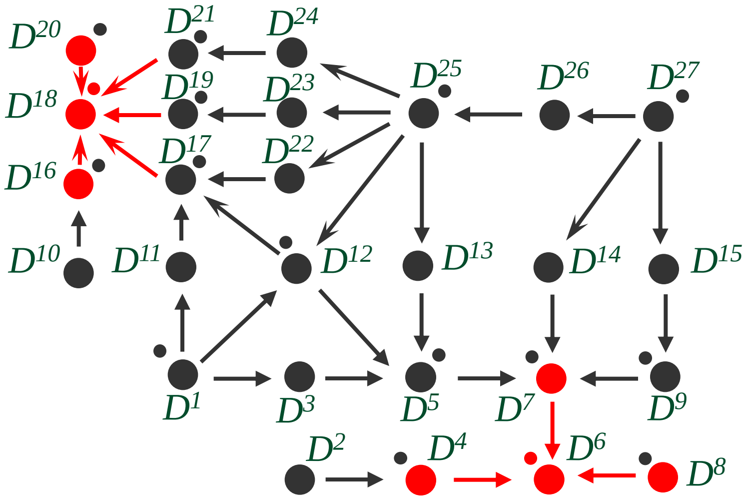
- Analysis of **steady states** and **limit cycles** of PL models
  - Attractor states in graph correspond (under certain conditions) to stable steady states of PL model

    Casey *et al.* (2006), *J. Math Biol.*, 52(1):27-56
  - Attractor cycles in graph correspond (under certain conditions) to stable limit cycles of PL model

    Glass and Pasternack (1978), *J. Math Biol.*, 6(2):207-23

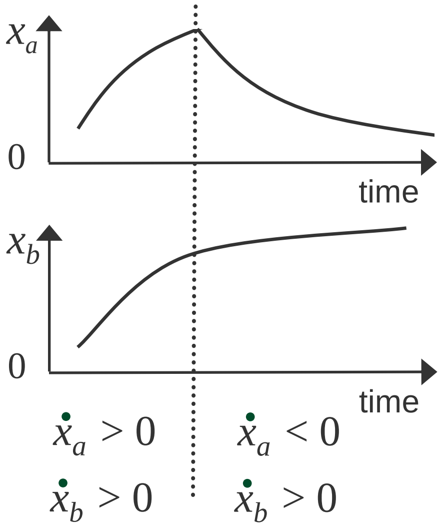
    Edwards (2000), *Physica D*, 146(1-4):165-99





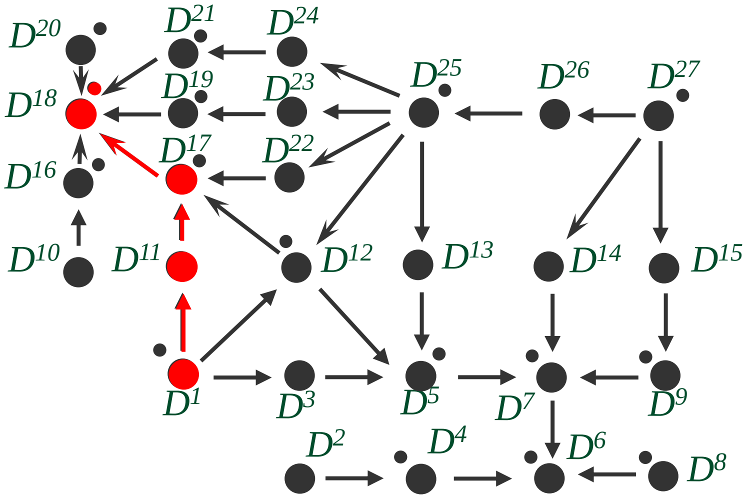
Use of state transition graph

- Paths in state transition graph represent predicted sequences of qualitative events
- **Model validation**: comparison of predicted and observed sequences of qualitative events



Concistency?

Yes



D^1: $\dot{x}_a > 0$, $\dot{x}_b > 0$

D^{17}: $\dot{x}_a < 0$, $\dot{x}_b > 0$

D^{18}: $\dot{x}_a = 0$, $\dot{x}_b = 0$

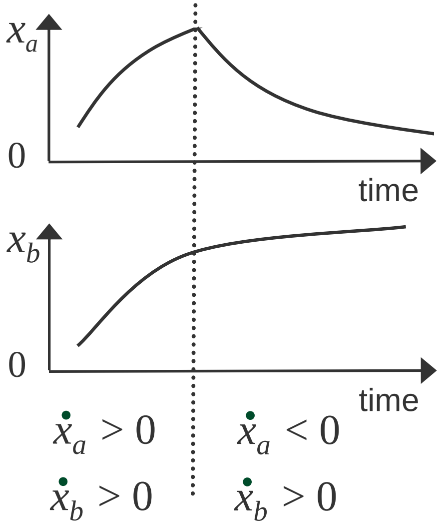
- Need for automated and efficient tools for model validation

Model validation by model checking

- Dynamic properties of system can be expressed in **temporal logic** (CTL)

There *E*xists a *F*uture state where $\dot{x}_a > 0$ and $\dot{x}_b > 0$
and starting from that state,
there *E*xists a *F*uture state where $\dot{x}_a < 0$ and $\dot{x}_b > 0$

$$\boldsymbol{EF}(\dot{x}_a > 0 \wedge \dot{x}_b > 0 \wedge \boldsymbol{EF}(\dot{x}_a < 0 \wedge \dot{x}_b > 0)\ )$$



- **Model checking** is automated technique for verifying that state transition graph satisfies temporal-logic statements

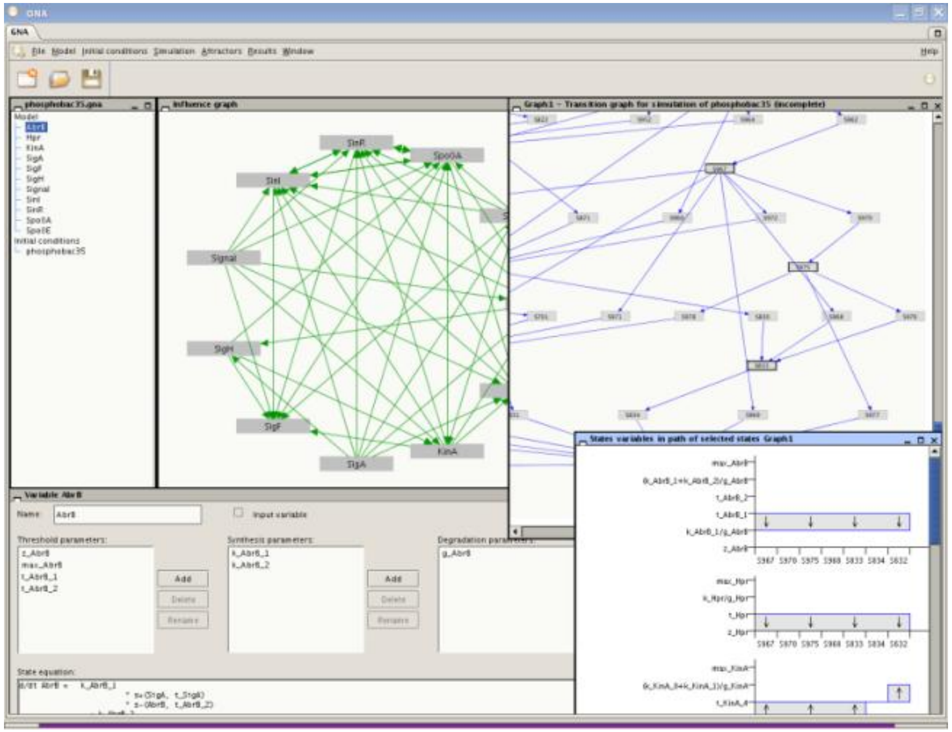
  Efficient computer tools available for model checking

Batt *et al.* (2005), *Bioinformatics*, 21(supp. 1): i19-i28

Genetic Network Analyzer (GNA)

- Qualitative analysis of PL models implemented in Java: **Genetic Network Analyzer (GNA)**

Distribution by
Genostar SA



de Jong *et al.* (2003),
Bioinformatics, 19(3):336-44

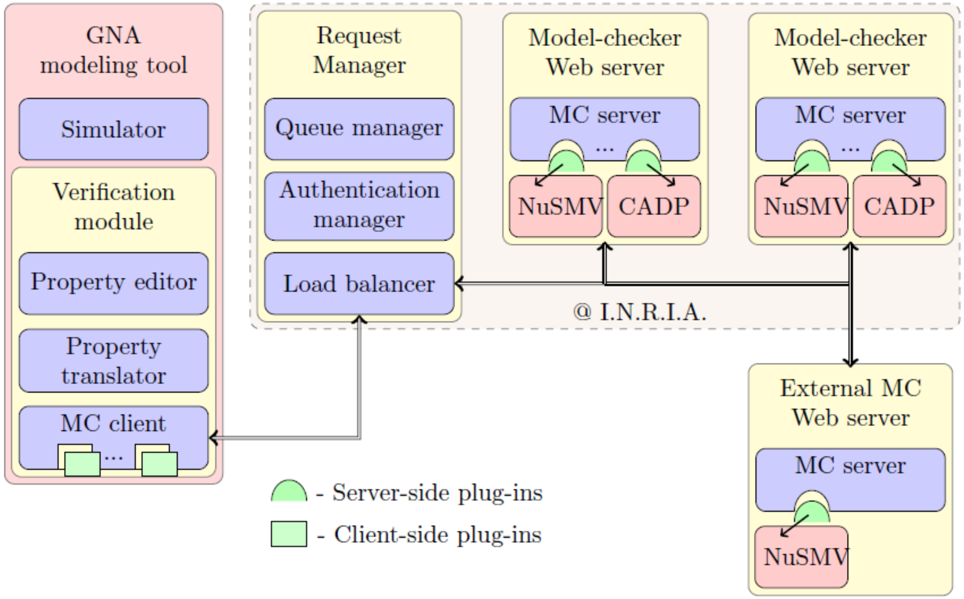
http://www-helix.inrialpes.fr/gna

Genetic Network Analyzer (GNA)

❖ Model-checking technology made available to GNA user

- Develop temporal logics tailored to biological questions

  Mateescu *et al.* (2010),
  Theor. Comput. Sci., in press



- Develop temporal-logic patterns patterns for frequently-asked modeling questions

  Monteiro *et al.* (2008), *Bioinformatics*, 24(16):i227-33

- Connect GNA to standard model checkers through a web-server connection

  Monteiro *et al.*, (2009), *BMC Bioinform.*, 10:450

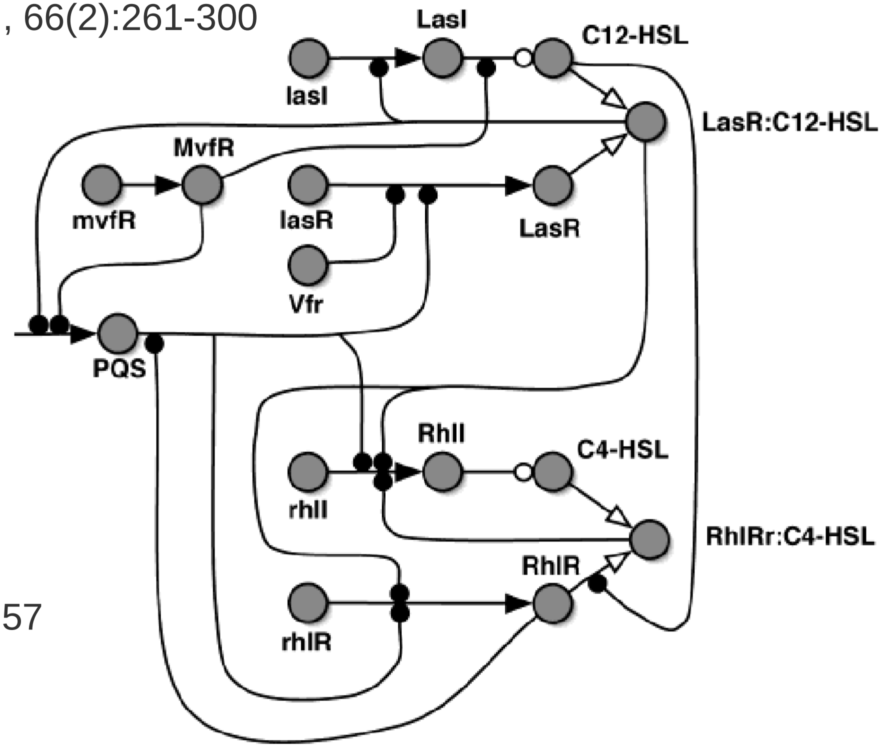
Analysis of bacterial regulatory networks

- Applications of qualitative simulation in bacteria:
  - Initiation of sporulation in *Bacillus subtilis*

    de Jong, Geiselmann *et al.* (2004), *Bull. Math. Biol.*, 66(2):261-300
  - Quorum sensing in *Pseudomonas aeruginosa*

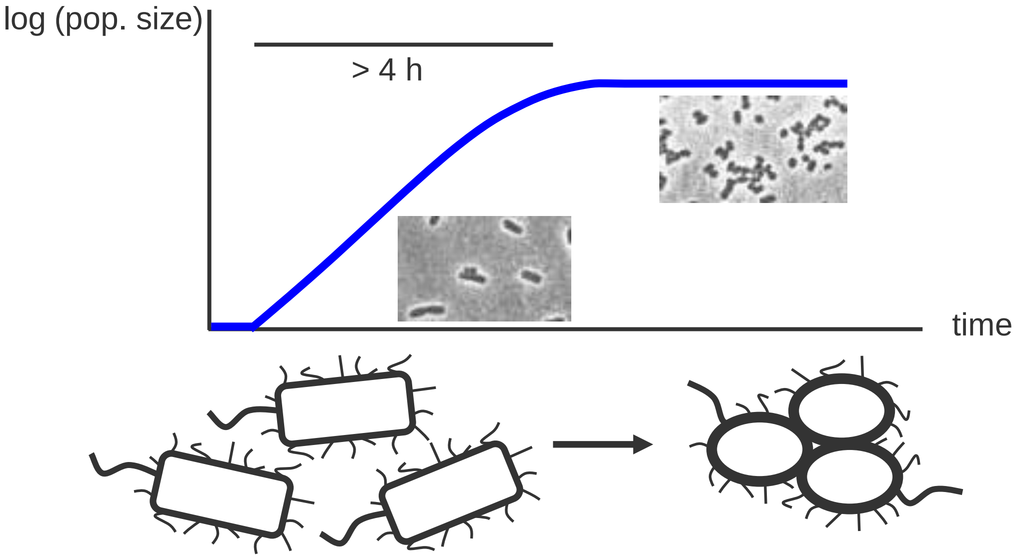
    Viretta and Fussenegger (2004), *Biotechnol. Prog.*, 20(3):670-8
  - Onset of virulence in *Erwinia chrysanthemi*

    Sepulchre *et al.* (2007), *J. Theor. Biol.*, 244(2):239-57



E. coli response to carbon starvation

- Response of *E. coli* to carbon starvation conditions: transition from **exponential phase** to **stationary phase**



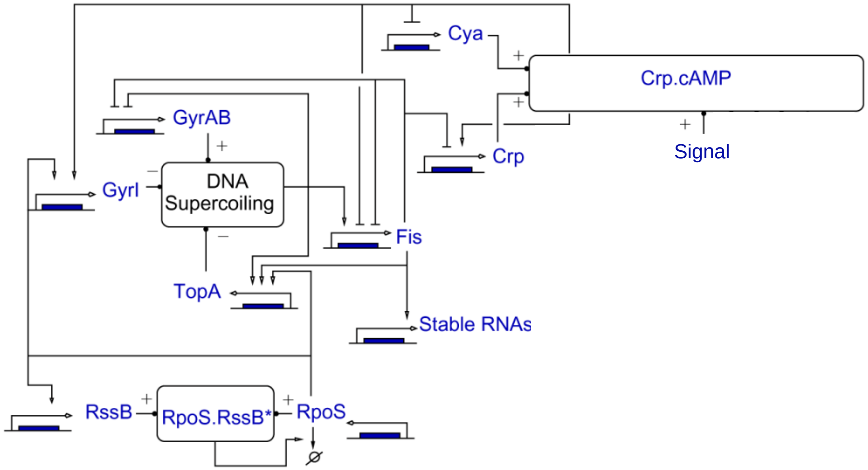
- Growth transition is accompanied by profound changes in gene expression, allowing cell to adjust its functioning to stress conditions

Storz and Hengge-Aronis (2000), *Bacterial Stress Responses*, ASM Press

Modeling of carbon starvation network

- Can we understand how gene regulatory network controls adaptation in response to carbon starvation?

    Network senses carbon source availability and **global regulators** coordinate adaptive response of bacteria



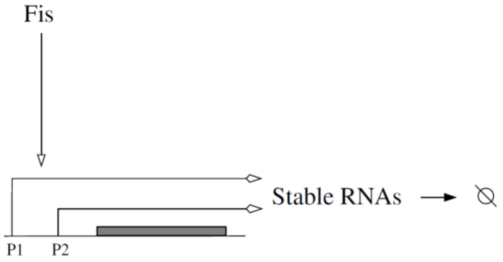
- Development of qualitative model of network

    Translation of network diagram into PL formalism (regulatory logic)

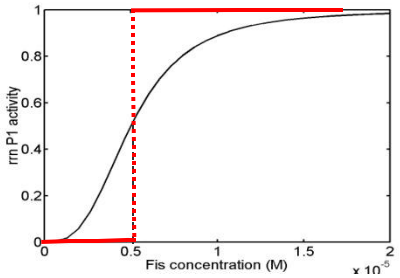
Ropers *et al.* (2006), *Biosystems*, 84(2):124-152

Development of PL model

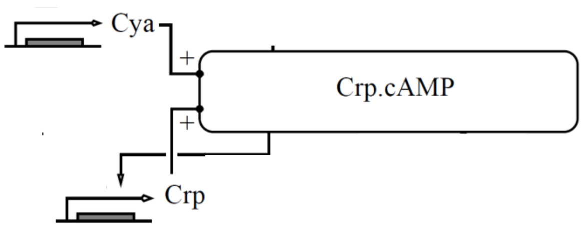
- Translation of network diagrams into PL models
  - Straightforward for direct interactions…

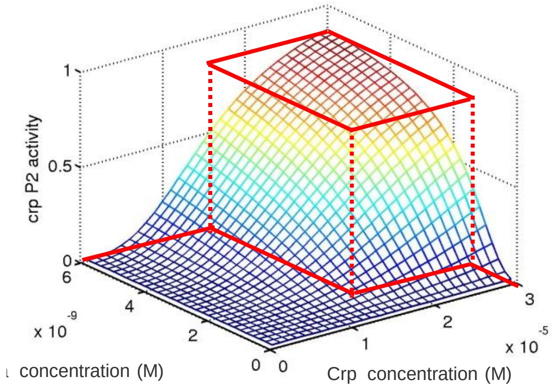


$$\dot{x}_n = \kappa_n^2 + \kappa_n^1\, s^+(x_f, \theta_f) - \gamma_n\, x_n$$



  - … but also possible for indirect interactions





$$\dot{x}_y = \kappa_y^1 + \kappa_y^2 - \gamma_y\, x_y$$
$$\dot{x}_c = \kappa_c^1 + \kappa_c^2\, s^+(x_c, \theta_c^1)\, s^+(x_y, \theta_y^1) - \gamma_c\, x_c$$

PL model of carbon starvation network

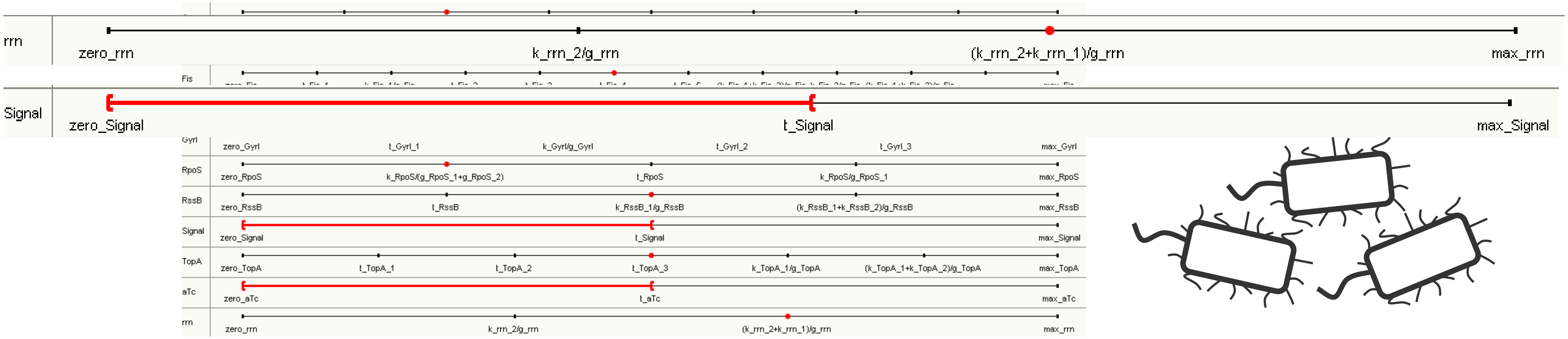
$$
\begin{aligned}
\dot{u}_s &= 0 \\
\dot{x}_y &= \kappa_y^1 + \kappa_y^2 \, (1 - s^+(x_c, \theta_c^2) \, s^+(x_y, \theta_y^2) \, s^+(u_s, \theta_s)) - \gamma_y \, x_y \\
\dot{x}_c &= \kappa_c^1 + \kappa_c^2 \, s^-(x_f, \theta_f^2) \, s^+(x_c, \theta_c^1) \, s^+(x_y, \theta_y^1) \, s^+(u_s, \theta_s) + \kappa_c^3 \, s^-(x_f, \theta_f^1) - \gamma_c \, x_c \\
\dot{x}_f &= \kappa_f^1 \, (1 - s^+(x_c, \theta_c^1) \, s^+(x_y, \theta_y^1) \, s^+(u_s, \theta_s)) \, s^-(x_f, \theta_f^6) \\
&\quad + \kappa_f^2 \, s^+(x_a, \theta_a^1) \, s^-(x_i, \theta_i^1) \, s^-(x_t, \theta_t^1) \, s^-(x_f, \theta_f^6) \\
&\quad \times (1 - s^+(x_c, \theta_c^1) \, s^+(x_y, \theta_y^1) \, s^+(u_s, \theta_s)) - \gamma_f \, x_f \\
\dot{x}_a &= \kappa_a \, (1 - s^+(x_a, \theta_a^2) \, s^-(x_i, \theta_i^2) \, s^-(x_t, \theta_t^2)) \, s^-(x_f, \theta_f^4) - \gamma_a \, x_a \\
\dot{x}_i &= \kappa_i \, s^+(x_c, \theta_c^1) \, s^+(x_y, \theta_y^1) \, s^+(u_s, \theta_s) \, s^+(x_o, \theta_o) - \gamma_i \, x_i \\
\dot{x}_o &= \kappa_o - (\gamma_o + k_7 \, s^+(x_b, \theta_b) \, s^-(u_s, \theta_s)) \, x_o \\
\dot{x}_b &= \kappa_b^1 + \kappa_b^2 \, s^+(x_o, \theta_o) - \gamma_b \, x_b \\
\dot{x}_t &= \kappa_t^1 \, s^+(x_a, \theta_a^3) \, s^-(x_i, \theta_i^3) \, s^-(x_t, \theta_t^3) \, s^+(x_f, \theta_f^5) + \kappa_t^2 \, s^+(x_o, \theta_o) - \gamma_t \, x_t \\
\dot{x}_n &= \kappa_n^1 \, s^+(x_f, \theta_f^3) + \kappa_n^2 - \gamma_n \, x_n
\end{aligned}
$$

- PL models supplemented with inequality constraints on parameter values

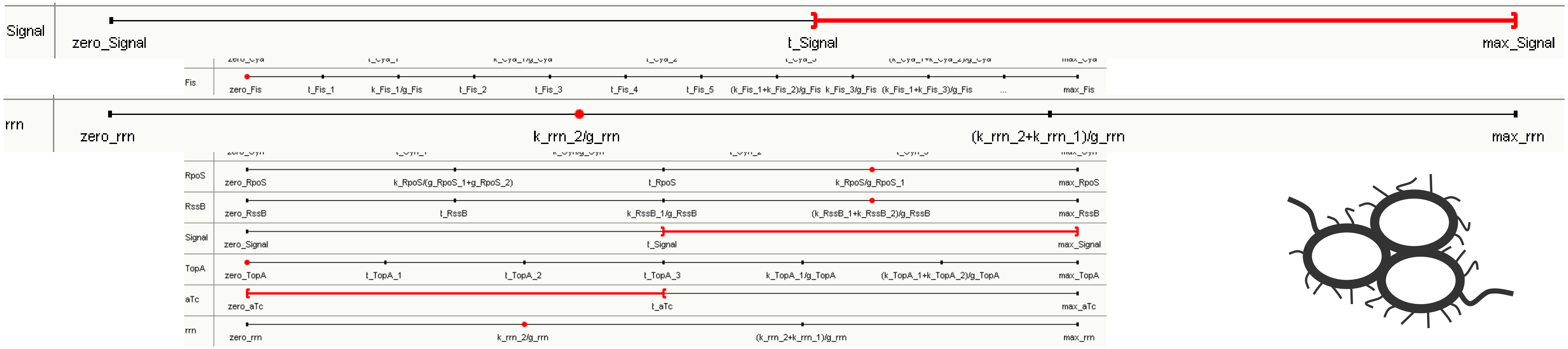
    Inequality constraints inferred from experimental literature

Attractors of stress response network

- Analysis of attractors of PA model: two **steady states**
  - **Stable** steady state, corresponding to exponential-phase conditions

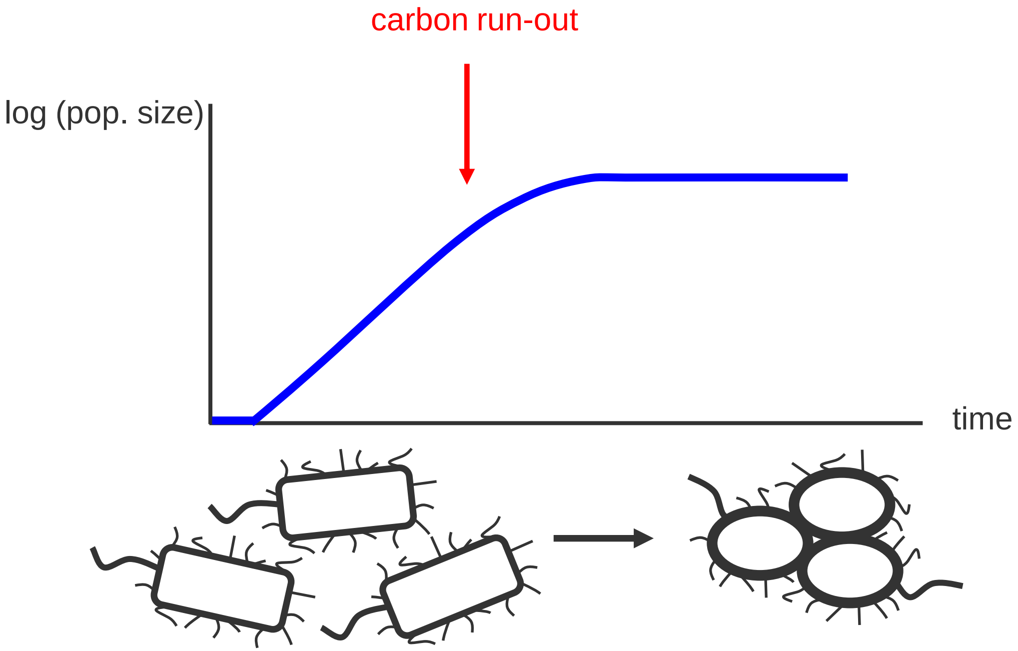


  - **Stable** steady state, corresponding to stationary-phase conditions



Transition to stationary phase

- Does model reproduce transition from exponential phase to stationary phase upon carbon starvation?

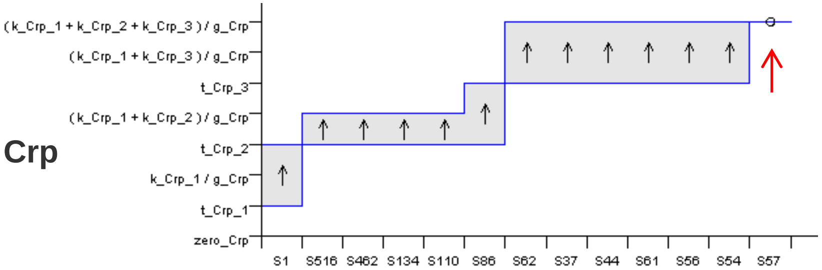


Qualitative simulation of network

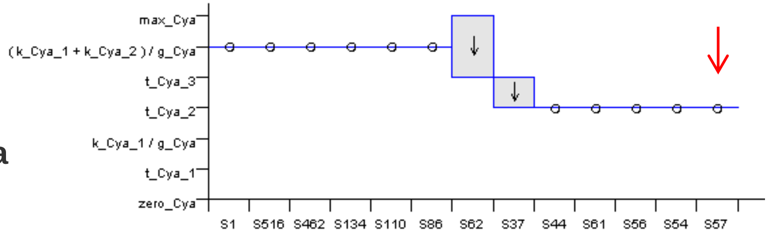
- Simulation of transition from exponential to stationary phase

  State transition graph with 851 states starting from exponential phase, all paths converge to stationary-phase steady state upon stress signal

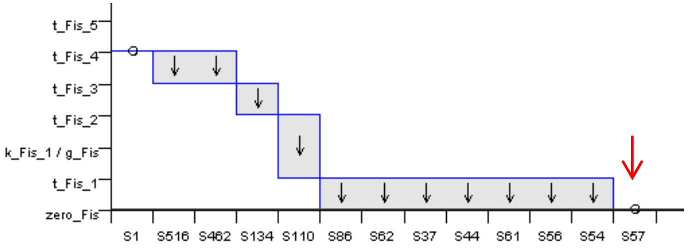
Crp



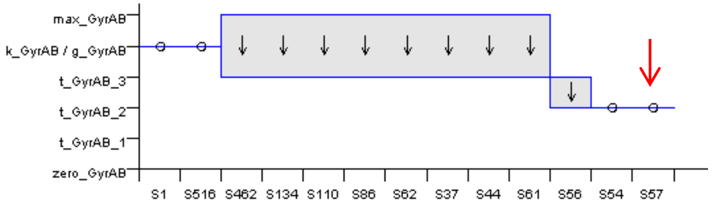
Cya



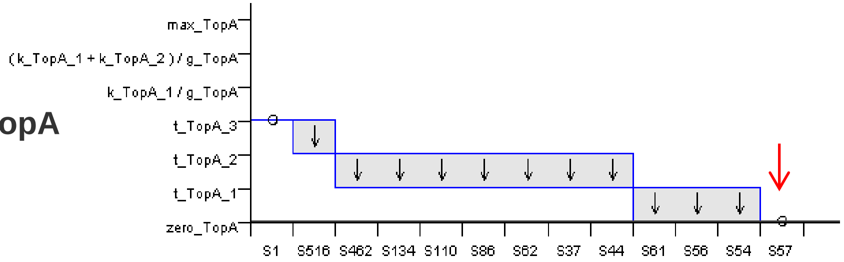
Fis



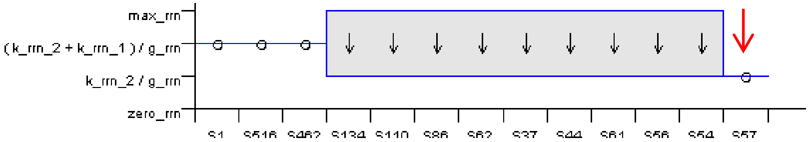
GyrAB



TopA

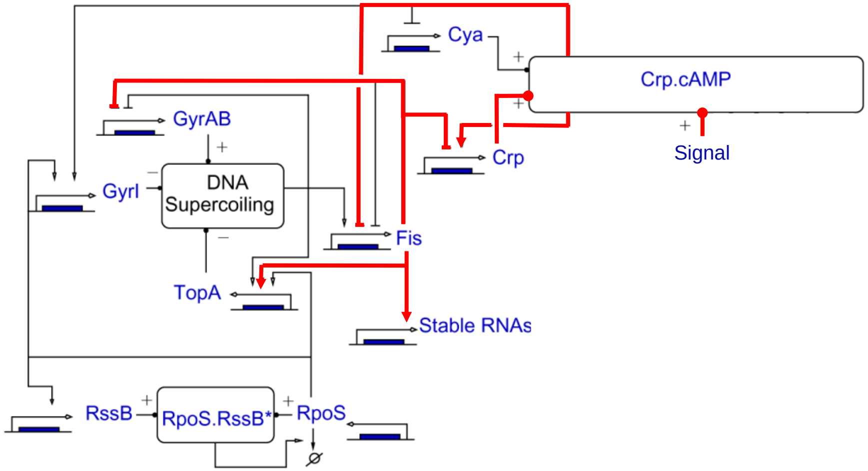


rrn



Hypothesis on carbon starvation response

- Sequence of **qualitative events** leading to adjustment of growth of cell after carbon starvation signal

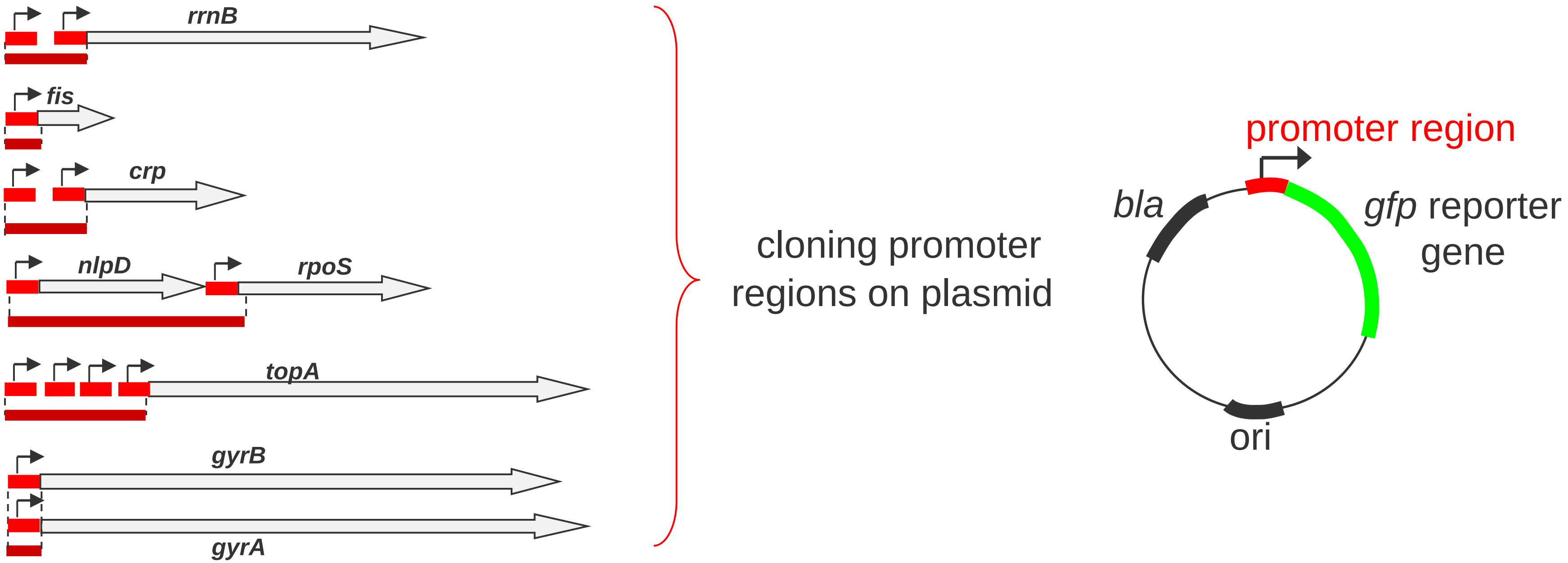


- Central role for **mutual inhibition** of Fis and Crp, the two major regulators of the cell

Cross inhibition functions as toggle switch, pulled by stress signal

Real-time monitoring of gene expression

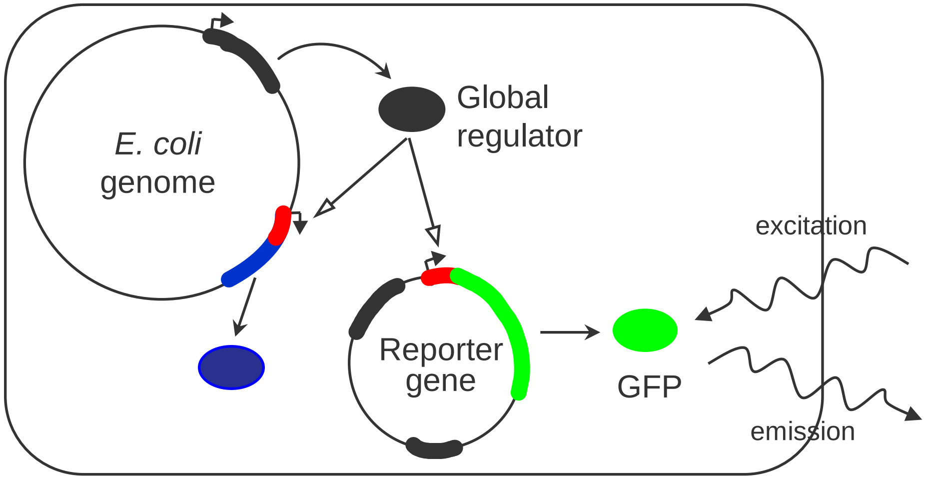
- Use of **reporter gene systems** to monitor gene expression *in vivo* and in real time
  - Fluorescent and luminescent reporters
  - Reporters on plasmid and in chromosome



cloning promoter regions on plasmid

Real-time monitoring of gene expression

- Integration of reporter gene systems into bacterial cell



Real-time monitoring of gene expression

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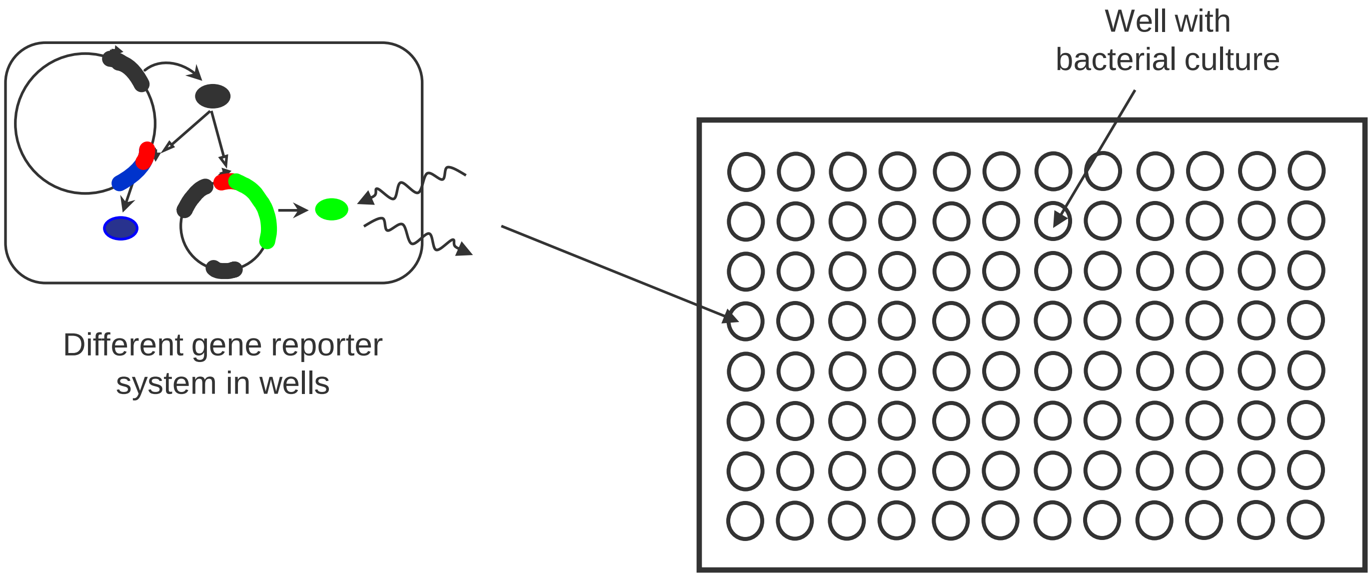


Different gene reporter system in wells

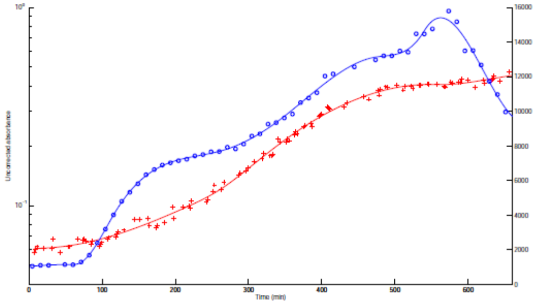
96-well microplate

- Use of automated microplate reader to monitor in parallel in single experiment expression of different reporter genes

Real-time monitoring of gene expression

- High-precision measurements of changes in gene expression on population level in response to environmental perturbations
  - Measurement of absorbance, fluorescence, luminescence
  - About 100 data points over an interval of 10 hours
- Treatment of raw data
  - Outlier detection, regression spline fitting with GCV, background substraction, confidence intervals via bootstrap, ...

Fluorescence and absorbance data

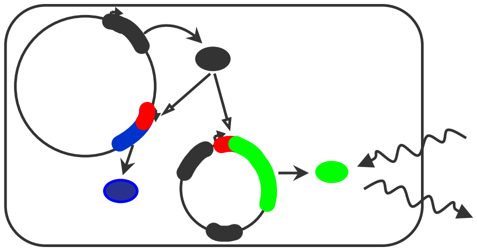


de Jong *et al*. (2010), *BMC Syst. Biol.*, 4:55

Real-time monitoring of gene expression

- Computation of biological quantities using kinetic models

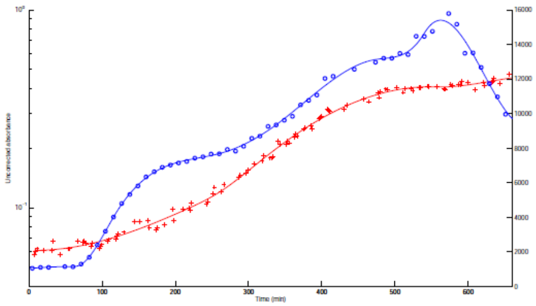
  Reporter protein concentrations and reporter synthesis rates (proportional to mRNA concentrations)



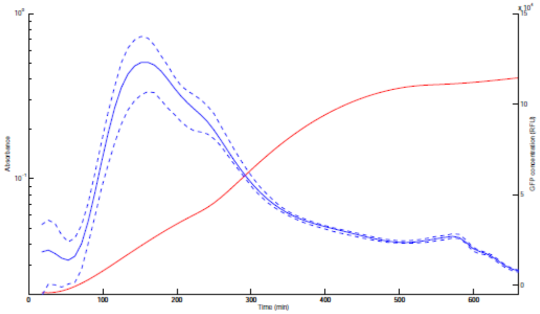
$$\frac{dn(t)}{dt} = \kappa_m\, f(t) - (\mu(t) + \gamma_n)\, n(t)$$

$$\frac{dq(t)}{dt} = \kappa_p\, n(t) - (\mu(t) + \gamma_q)\, q(t)$$

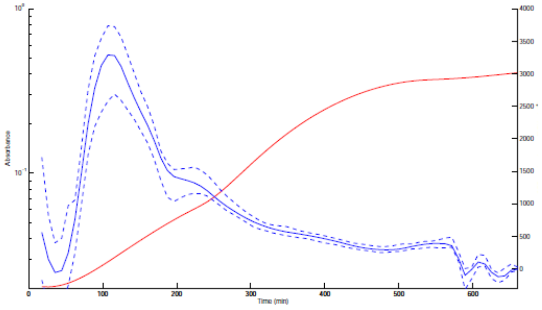
$$\frac{dr(t)}{dt} = \kappa_r\, (q(t) - r(t)) - (\mu(t) + \gamma_q)\, r(t)$$



Fluorescence and absorbance data



Fis reporter concentration

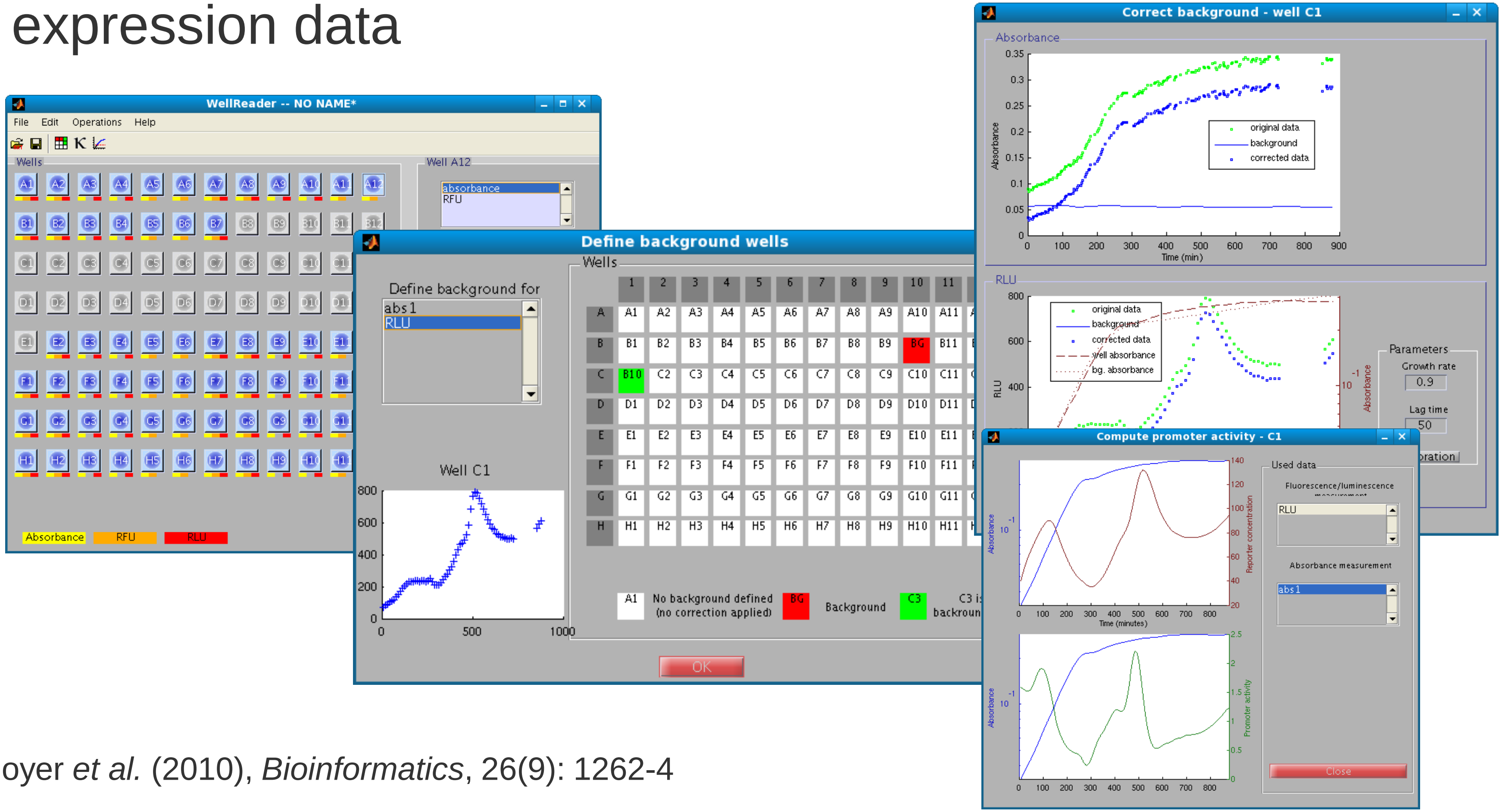


fis reporter mRNA concentration

de Jong *et al*. (2010), *BMC Syst. Biol.*, 4:55

Real-time monitoring of gene expression

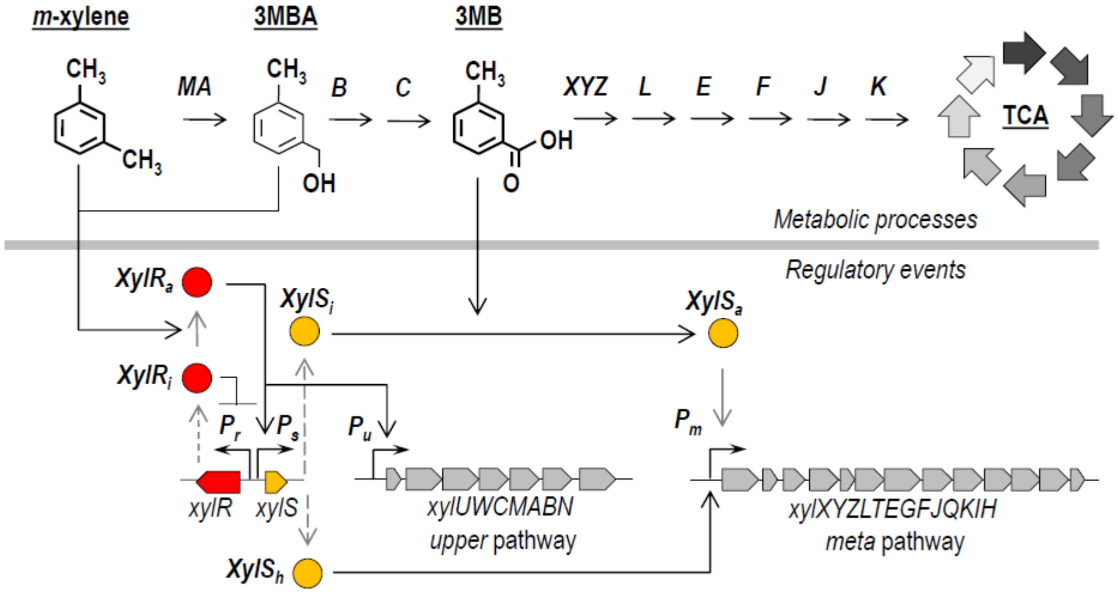
- **Wellreader**: Matlab program for analysis of reporter gene expression data



Boyer *et al.* (2010), *Bioinformatics*, 26(9): 1262-4

Biodegradation of pollutants by *P. putida*

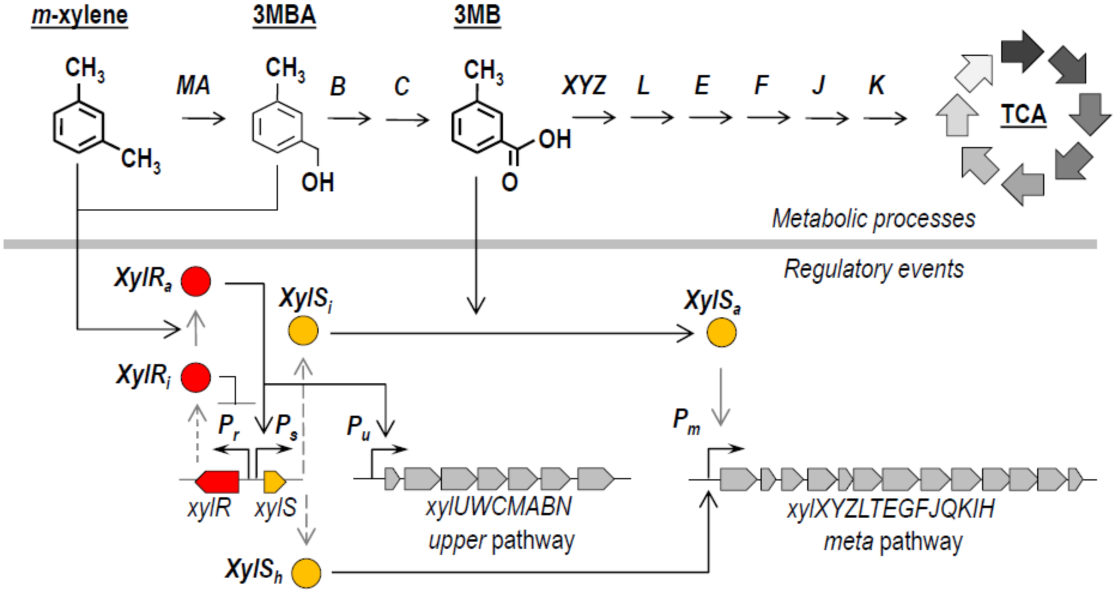
- Soil bacterium *Pseudomonas putida* mt-2 is archetypal model for environmental biodegradation of aromatic pollutants
  - TOL network involved in degradation of *m*-xylene to intermediates for central carbon metabolism



Rocha-Silva *et al.* (2011), *Environ. Microbiol.*, in press

Role of regulators of TOL network

- What is the role of the central, plasmid-encoded regulators XylR and XylS?



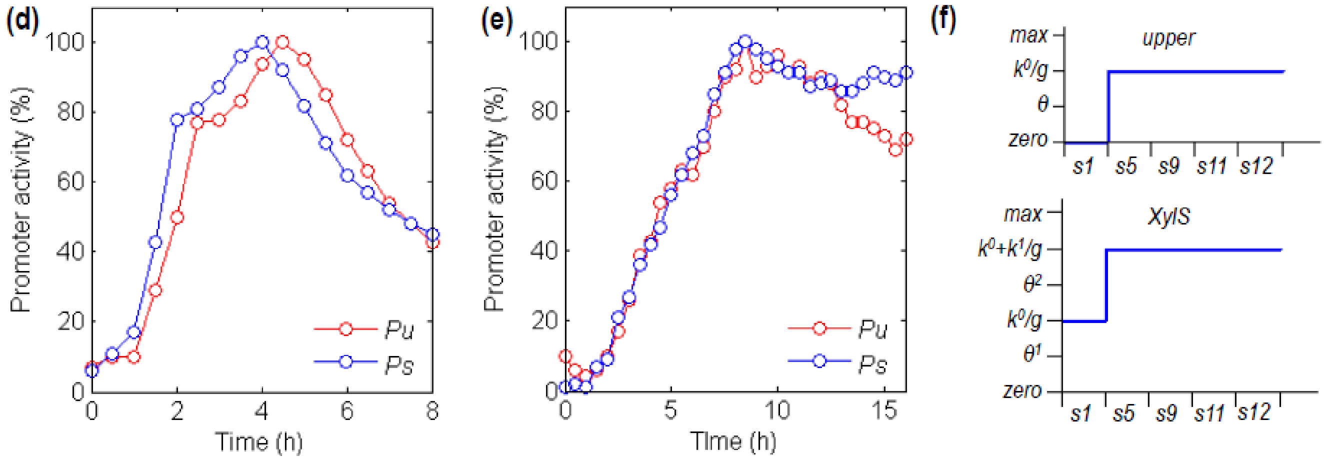
- Development of PL model of TOL network

Translation of network diagram into regulatory logic and PL models

Rocha-Silva *et al.* (2011), *BMC Syst. Biol*., in press

Role of regulators of TOL network

- Validation of model by testing predictions under different perturbation conditions (mutants, metabolic inducers, …)



Rocha-Silva *et al.* (2011), *BMC Syst. Biol.*, in press

- Plasmid-encoded regulators of TOL network act as **regulatory firewall**

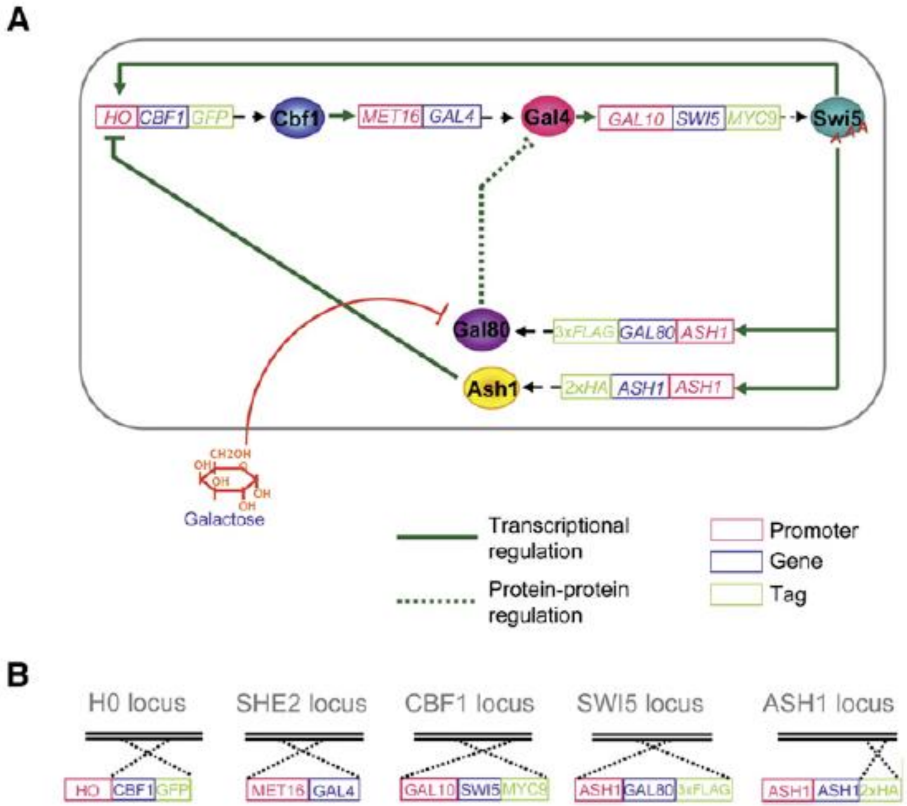
  Prevent toxic *m*-xylene and its biodegradation intermediates from intervening with indigenous metabolic pathways

IRMA: synthetic network in yeast

- **IRMA**: synthetic network in yeast consisting of interlocked positive and negative feedback loops

    Networks functions independently from host cell

- Network can be externally controlled by growing cells in glucose or galactose

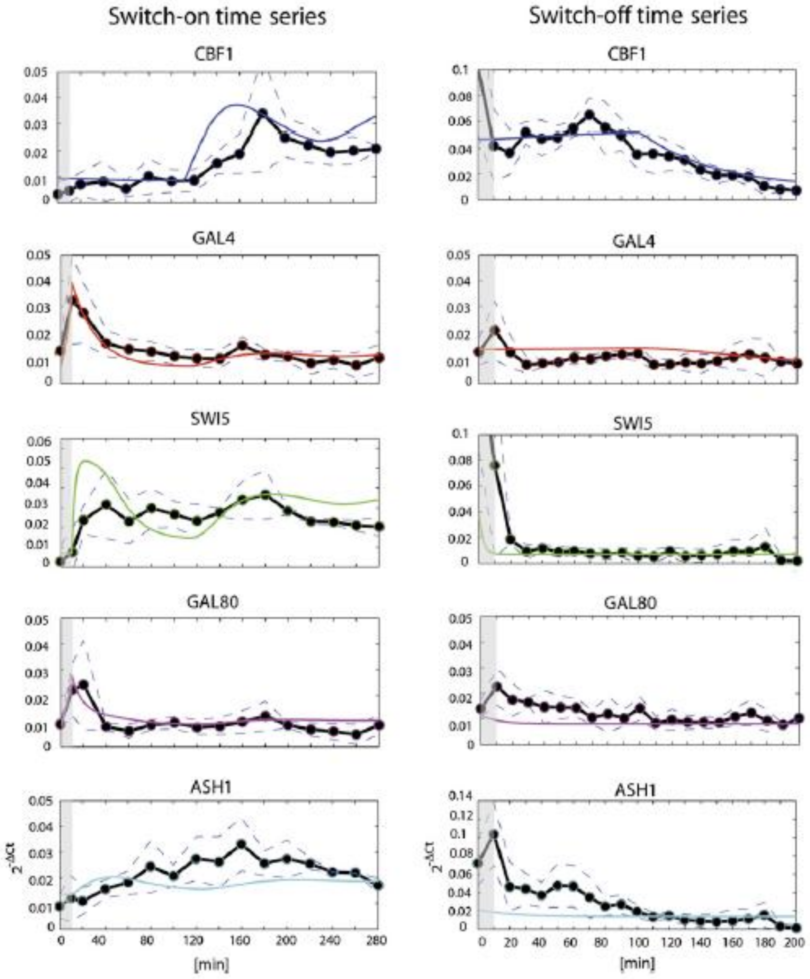


Cantone *et al.* (2009), *Cell*, 137(1):172-81

IRMA: synthetic network in yeast

- IRMA proposed as a benchmark for modeling and identification approaches
- IRMA dynamics measured over time in galactose (switch-on) and glucose (switch-off)

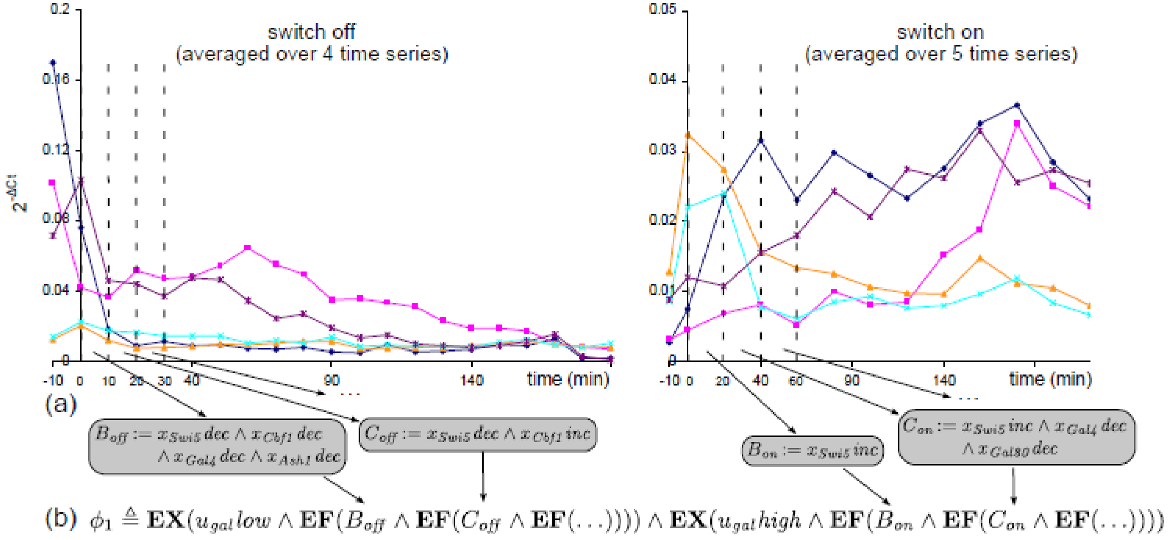
  Quantitative RT-PCR
- **Question**: are measured dynamics consistent with constructed network structure?



Cantone *et al.* (2009), *Cell*, 137(1):172-81

Test of consistency structure-dynamics

- Development of (unparametrized) PL model representing network structure
- Approach to test consistency between network structure and data based on automated parameter constraint search:
  - Generate temporal logic formulae encoding observed network dynamics



Batt *et al.* (2010), *Bioinformatics*, 26(18):i603-10

Test of consistency structure-dynamics

- Development of (unparametrized) PL model representing network structure
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  - Generate temporal logic formulae encoding observed network dynamics
  - Test if there are any parametrizations of PL model satisfy temporal logic formulae

	Symbolic state space and symbolic parameter space		Symbolic state space and explicit parameter space	
Property	Existence of parametrization	Parametrization*	Number of parametrizations	Parametrization*
ϕ_1: averaged time-series	Yes (49 s)	$\frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^g_{Swi5} < \theta^c_{Swi5} < \theta^a_{Swi5} < \frac{\kappa^0_{Swi5}+\kappa_{Swi5}}{\gamma_{Swi5}} \wedge \frac{\kappa^0_{Gal80}}{\gamma_{Gal80}} < \frac{\kappa^0_{Gal80}+\kappa_{Gal80}}{\gamma_{Gal80}} < \theta_{Gal80}$	12 (925 s)	$\frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^c_{Swi5} < \theta^a_{Swi5} < \frac{\kappa^0_{Swi5}+\kappa_{Swi5}}{\gamma_{Swi5}} \wedge (\theta_{Gal80} < \frac{\kappa^0_{Gal80}}{\gamma_{Gal80}} \wedge \frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^g_{Swi5} < \frac{\kappa^0_{Swi5}+\kappa_{Swi5}}{\gamma_{Swi5}} \vee \frac{\kappa^0_{Gal80}}{\gamma_{Gal80}} < \theta_{Gal80} < \frac{\kappa^0_{Gal80}+\kappa_{Gal80}}{\gamma_{Gal80}} \wedge \frac{\kappa^0_{Swi5}}{\gamma_{Swi5}} < \theta^g_{Swi5} \vee \frac{\kappa^0_{Gal80}+\kappa_{Gal80}}{\gamma_{Gal80}} < \theta_{Gal80})$

* All parametrizations additionally include $\kappa^1_{Cbf1}/\gamma_{Cbf1} < \theta_{Cbf1} < (\kappa^1_{Cbf1} + \kappa^2_{Cbf1})/\gamma_{Cbf1} \wedge \kappa^0_{Gal4}/\gamma_{Gal4} < \theta_{Gal4} < (\kappa^0_{Gal4} + \kappa_{Gal4})/\gamma_{Gal4} \wedge \kappa^0_{Ash1}/\gamma_{Ash1} < \theta_{Ash1} < (\kappa^0_{Ash1} + \kappa_{Ash1})/\gamma_{Ash1}$.

Test of consistency structure-dynamics

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  - Generate temporal logic formulae encoding observed network dynamics
  - Test if there are any parametrizations of PL model satisfy temporal logic formulae
  - Analyze parametrizations for biological plausibility

« Activation threshold of *CBF1* by Swi5 higher than activation threshold of *ASH1* »: confirmed by independent experimental data

Batt *et al.* (2010), *Bioinformatics*, 26(18):i603-10

Test of consistency structure-dynamics

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  - Generate temporal logic formulae encoding observed network dynamics
  - Test if there are any parametrizations of PL model satisfy temporal logic formulae
  - Analyze parametrizations for biological plausibility
- Automated approach for testing coherence between network structure and data based on model-checking techniques

  Symbolic encoding of model, dynamics and properties to make problem feasible

Perspectives: towards quantitative models

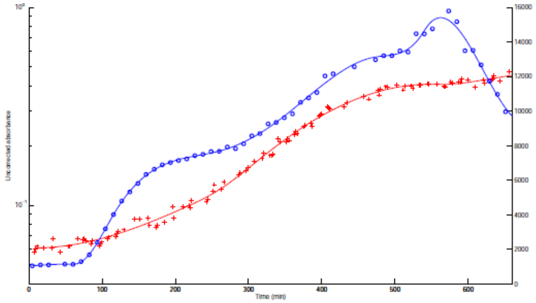
- Qualitative models can help **understand basic principles underlying dynamics** of complex gene regulatory networks

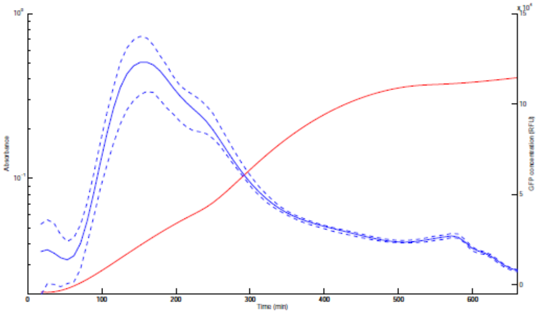
  Models provide a coarse-grained picture of the dynamics, but capture the regulatory logic of the network of interactions

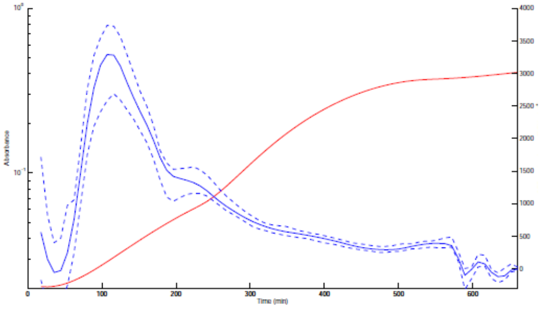
- Biological validation of qualitative models has a long history, and **experimental validation** of specific predictions is increasingly becoming possible
  - New **measurement techniques**: fluorescent reporter genes, quantitative proteomics , metabolomics, …
  - New ways to **control biological experiments**: overexpression plasmids, microfluidics, …

Perspectives: towards quantitative models

- Quality of data produced by new experimental techniques makes it increasingly realistic to work with fully quantitative ODE models
  - More accurate representation of biochemical mechanisms
  - More precise predictions
- Perspective raises new methodological challenges: parameter estimation in large nonlinear ODE models







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  - New **measurement techniques**: fluorescent reporter genes, plate readers (cell populations) and microscopes (individual cells), quantitative proteomics and metabolomics, …
  - New ways to **control biological systems**: overexpression plasmids, microfluidics, synthetic biology, …

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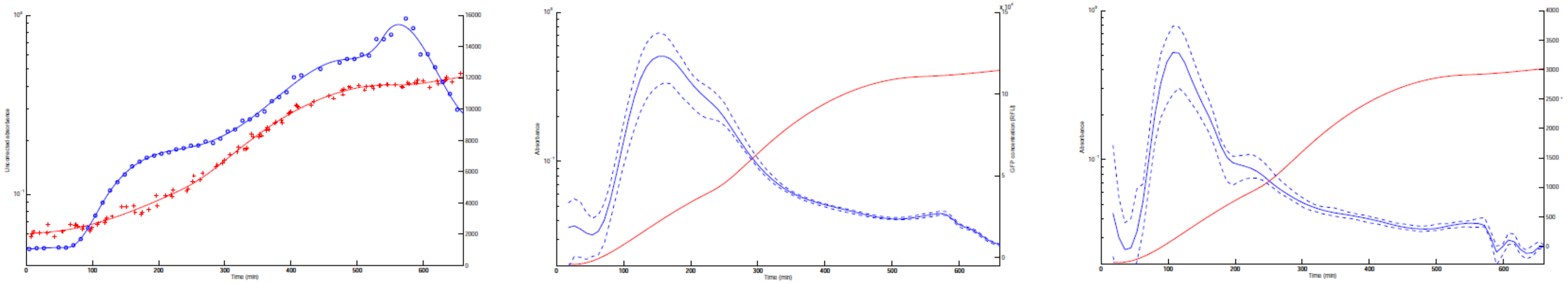
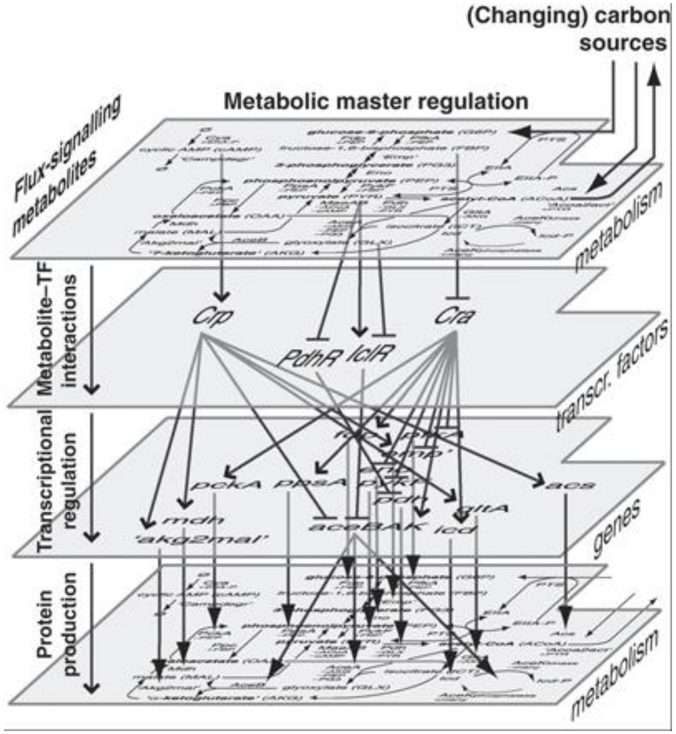
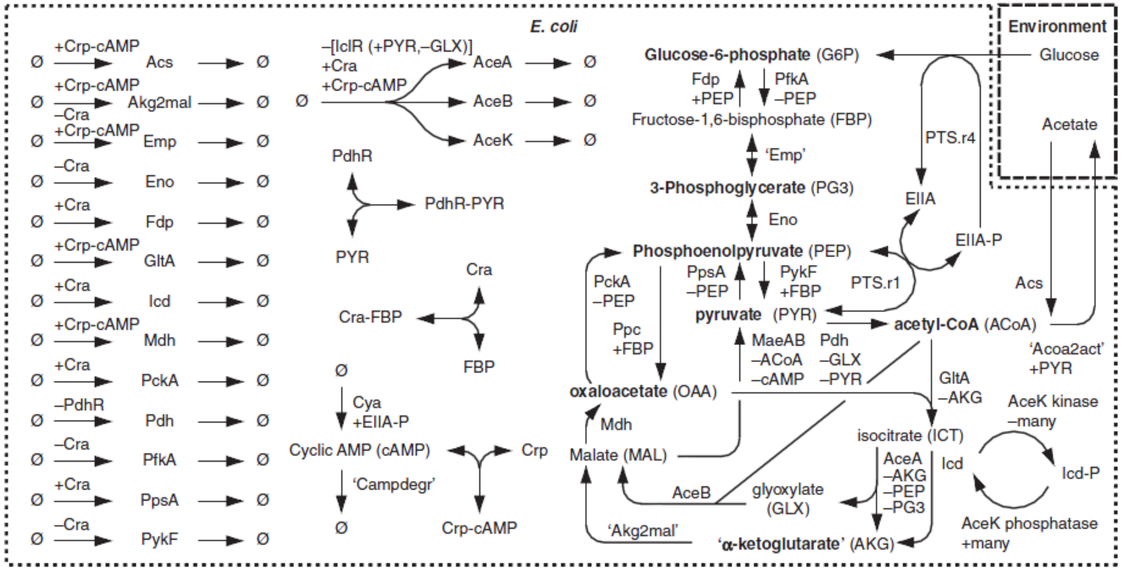


Perspectives: towards quantitative models

- Coupling of global regulation of transcription with carbon metabolism

Modeling of glucose-acetate shift in *E. coli*





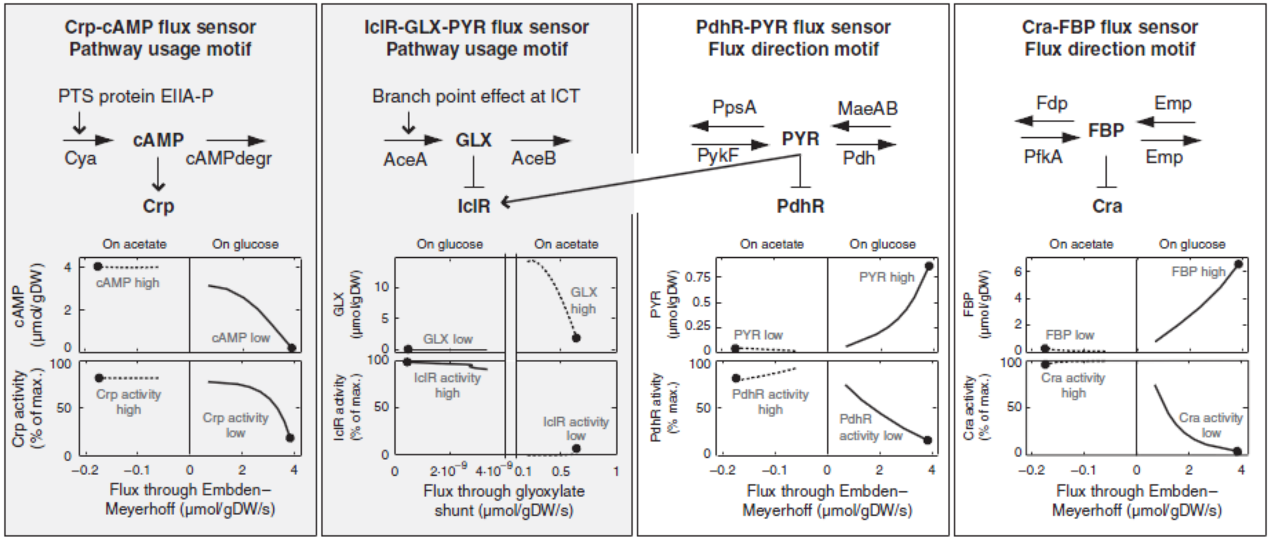
Kotte *et al*. (2010), *Mol. Syst. Biol*., 6: 355

Perspectives: towards quantitative models

- Kinetic model with 47 variables and 193 parameters

  Parameters estimated from published experimental steady-state data sets for balanced growth on either glucose or acetate

- Analysis of model shows that adaptation to change in carbon source is achieved by distributed sensing of intracellular fluxes



Kotte *et al*. (2010), *Mol. Syst. Biol*., 6: 355

Perspectives: towards quantitative models

- No algorithms that guarantee globally optimal solution for parameter estimation in nonlinear models

  Evolutionary algorithms, simulated annealing, genetic algorithms, …

- Parameter estimation demands experimental data of sufficient quantity and quality

  Common problems: noise, sampling density, unobserved variables, …

Ashyraliyev et al. (2009), *FEBS J.*, 276:886-902

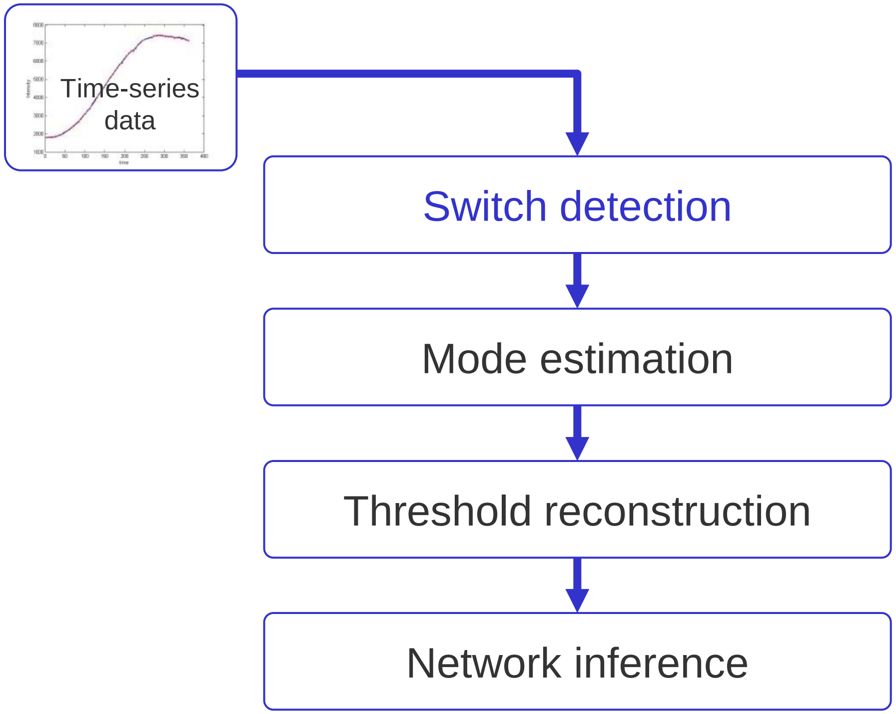
van Riel (2006), *Brief. Bioinform.*, 7(4):364–74

- Moreover, models of regulatory networks may be non-identifiable by principle, but …

  … even partially identifiable models may yield interesting results!

Perspectives: network identification

- Adaptation of methods for hybrid-systems identification to PL models of genetic regulatory networks



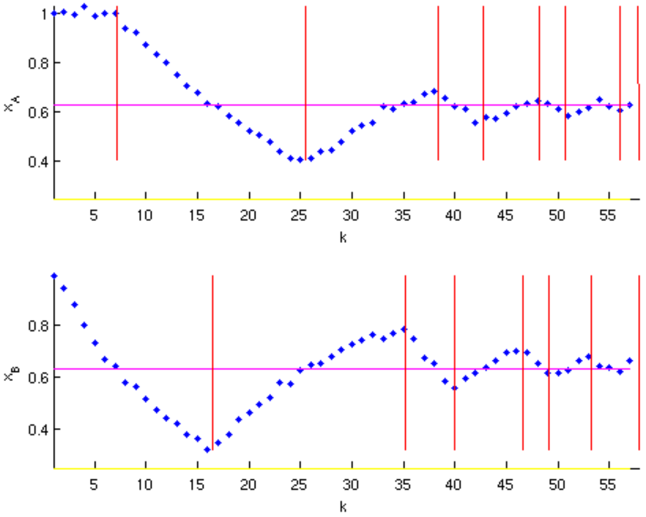
Time-series data

Switch detection

Mode estimation

Threshold reconstruction

Network inference



Drulhe *et al.* (2008), *IEEE Trans. Autom. Control*, 53(1):153-65
Porreca *et al.* (2008), *J. Comput. Biol.*, 15(10):1365-80

Perspectives: network identification

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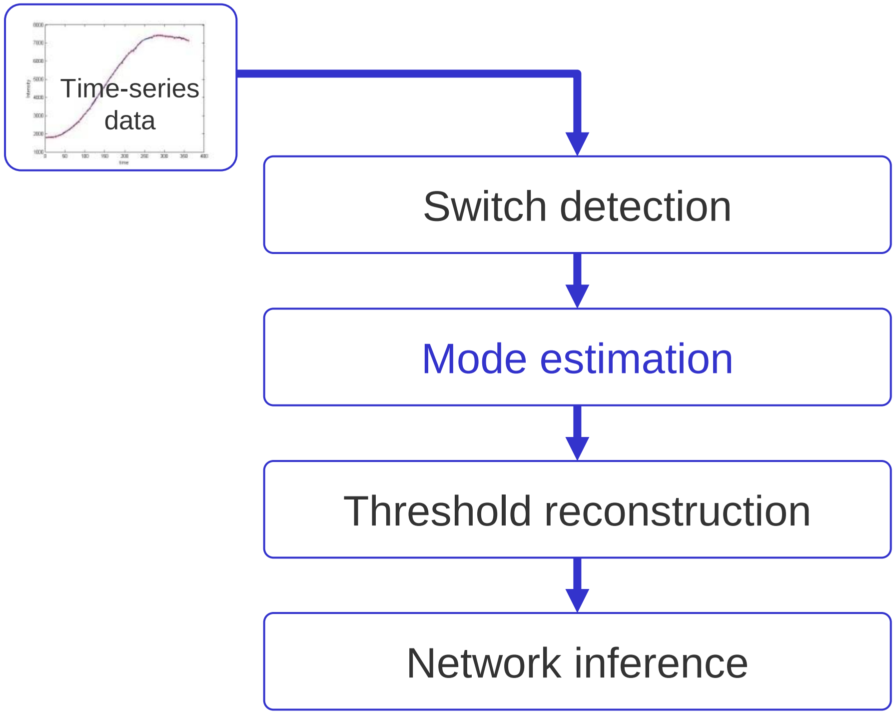
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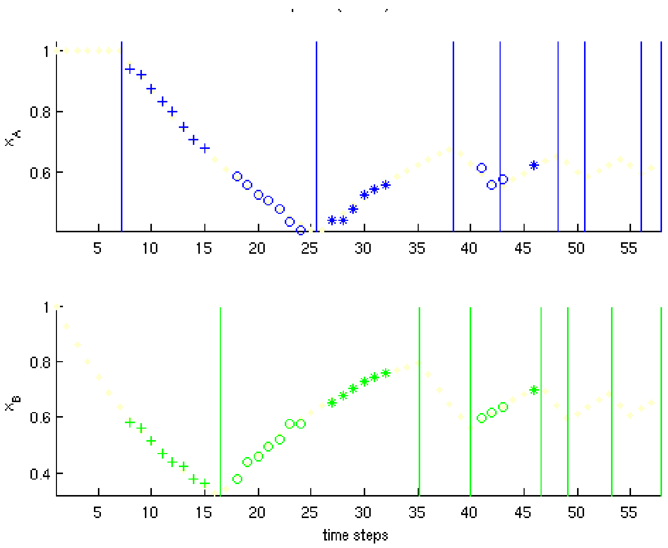

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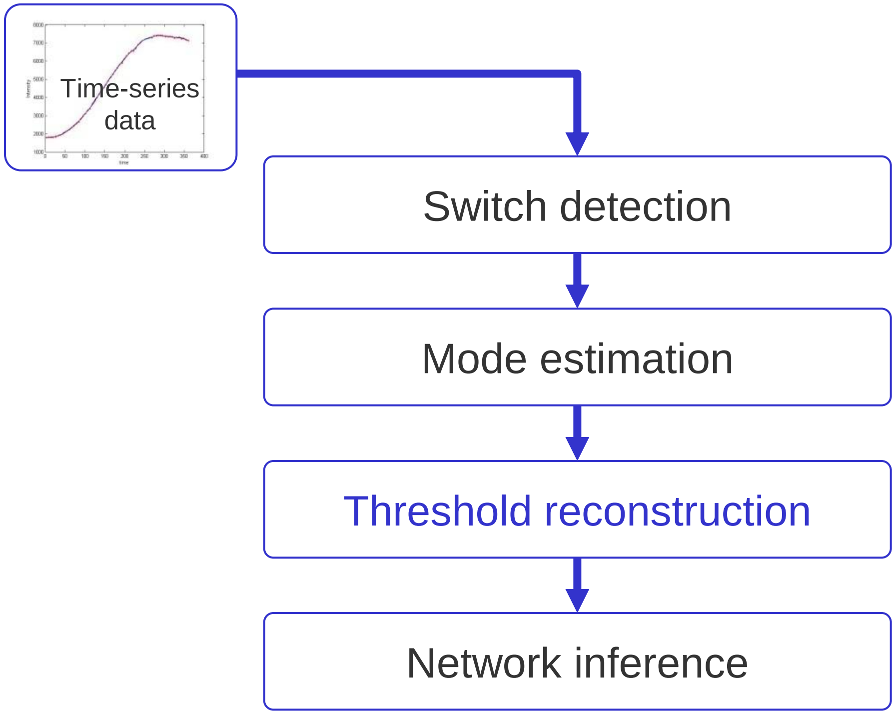

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Porreca *et al.* (2008), *J. Comput. Biol.*, 15(10):1365-80

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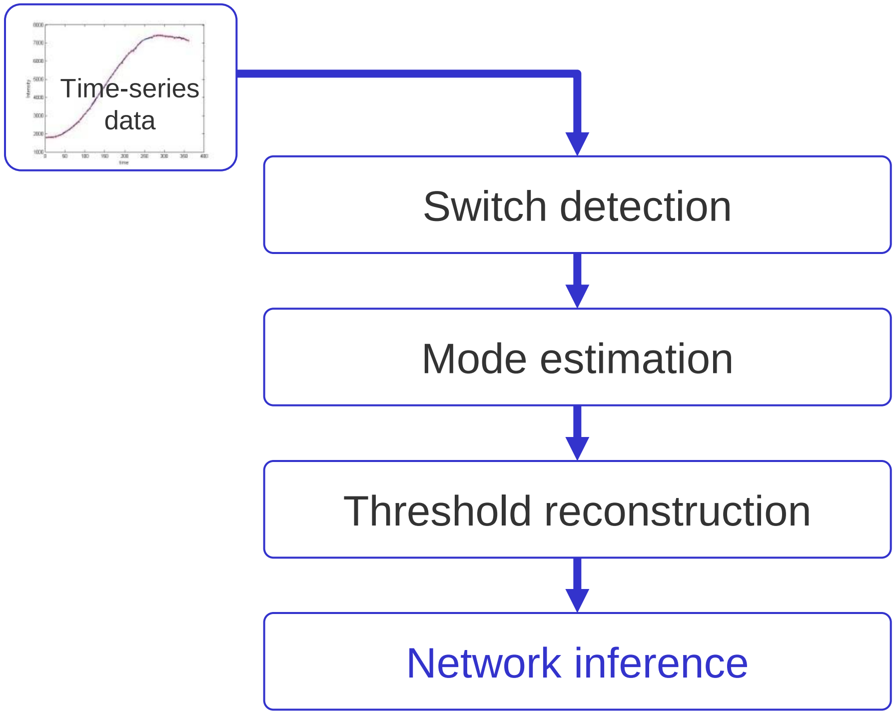
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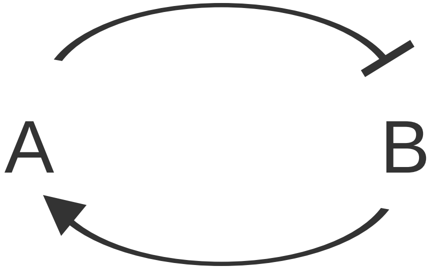

Drulhe *et al.* (2008), *IEEE Trans. Autom. Control*, 53(1):153-65
Porreca *et al.* (2008), *J. Comput. Biol.*, 15(10):1365-80

Perspectives: towards controller networks

- Instead of understanding how naturally occuring regulatory networks control cellular processes (**systems biology**)…
- … one could try to (re)design synthetic regulatory networks for controlling cellular processes (**synthetic biology**)
- Reprogramming natural pathways by means of synthetic toggle switch

  SOS pathway from *E. coli* (DNA damage) and transgenic pathway from *V. fischeri* (quorum sensing)

Kobayashi *et al.* (2004), *Proc. Natl. Acad. Sci. USA*, 101(22):8414–9

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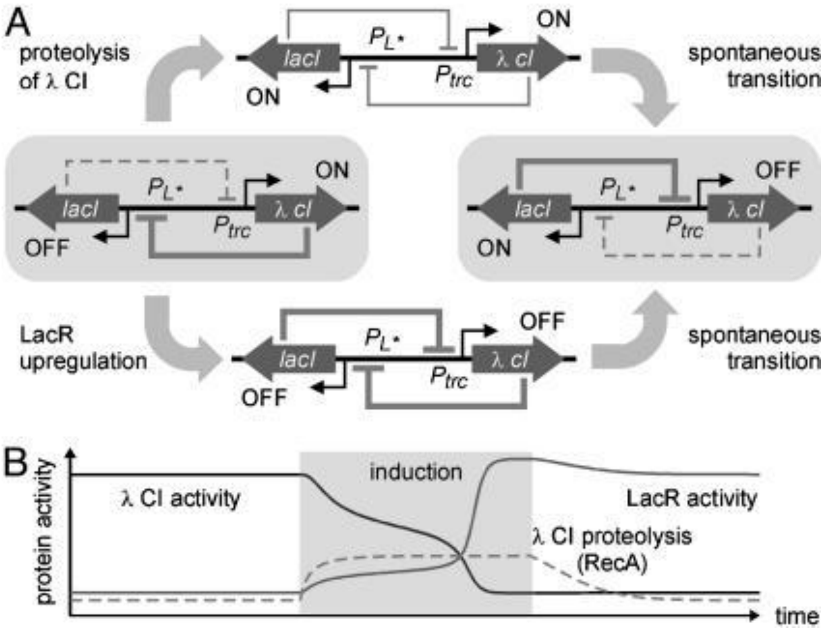
- Reprogramming natural pathways by means of synthetic toggle switch

  SOS pathway from *E. coli* (DNA damage) and transgenic quorum sensing pathway from *V. fischeri* (quorum sensing)



Kobayashi *et al.* (2004), *Proc. Natl. Acad. Sci. USA*, 101(22):8414–9

Perspectives: towards controller networks

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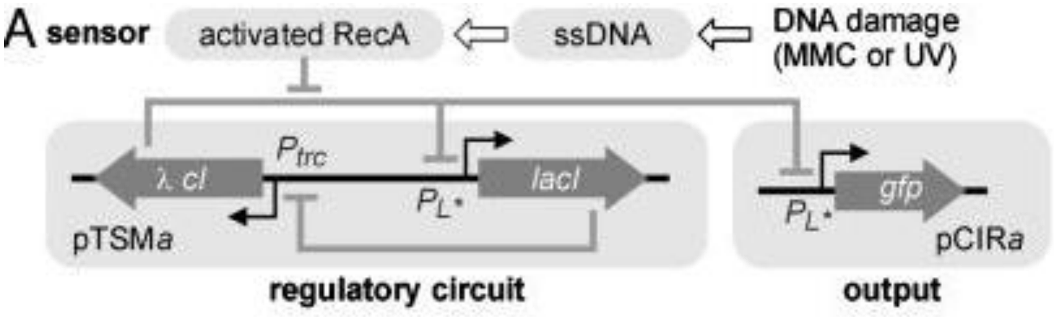

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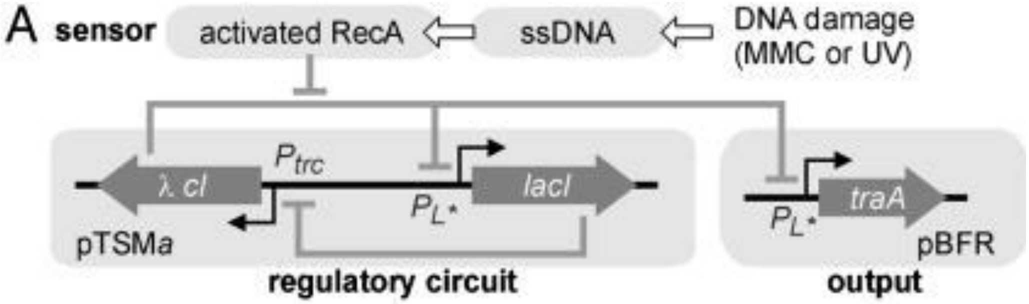


  Strain produces biofilm when DNA damage is induced by UV light or by antibiotic (mitomycin)

Kobayashi *et al.* (2004), *Proc. Natl. Acad. Sci. USA*, 101(22):8414–9

Perspectives: towards controller networks

- Instead of understanding how naturally occuring regulatory networks control cellular processes (**systems biology**)…
- … one could try to (re)design synthetic regulatory networks for controlling cellular processes (**synthetic biology**)
- New and very active domain
  - Student competition iGEM
- Potential applications:
  - Biotechnology (biofuels, …)
  - Medicine (viruses targeting cancer cells, …)

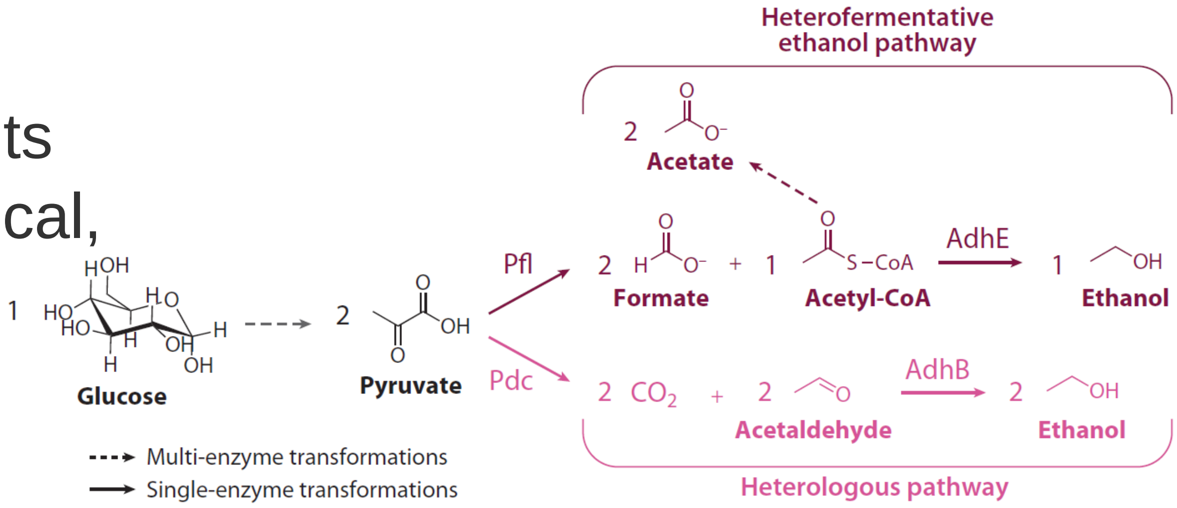
E. coli and biofuel production

- Can *E. coli* cells be engineered to convert sugars into fuels at high yield and productivity?

  Fermentable sugars obtained from agricultural waste (cellulose, …)

- Redesign *E. coli* cell by including pathways from other organisms and modifying regulatory mechanisms

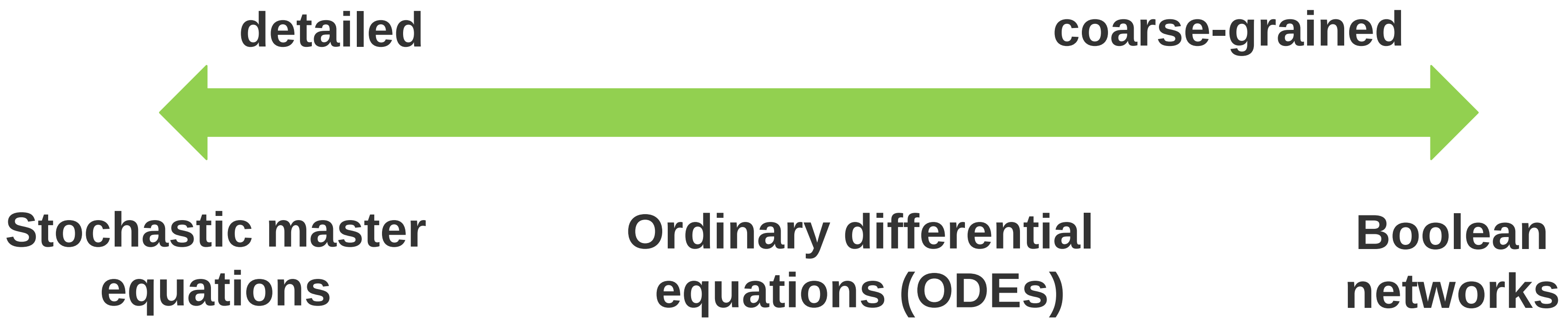
- Development of biorefineries only in its infancy, many technical, economical, and environmental challenges



Liu, Khoshla (2010), *Annu. Rev. Genet.*, 44:53-69

Conclusions

- Variety of modeling formalisms and computer tools for modeling of gene regulatory networks exist
  - Formalisms built on different modeling assumptions, suitable for answering different questions
  - Choice of modeling formalism is choice of tool, not necessarily choice of world view



detailed

coarse-grained

Stochastic master equations

Ordinary differential equations (ODEs)

Boolean networks

Conclusions

- Modeling of genetic regulatory networks in bacteria often hampered by lack of information on parameter values
- Use of coarse-grained PL models that provide reasonable approximation of dynamics
- Mathematical methods and computer tools for analysis of qualitative dynamics of PL models

  Weak information on parameter values (inequality constraints)
- Use of PL models may gain insight into functioning of large and complex networks
- PL models provide first idea of qualitative dynamics that may guide modeling by means of quantitative models

Some challenges for modelers

- Upscaling of analysis to large networks of dozens or even hundreds of genes, proteins, metabolites, …
  - Model reduction, qualitative models, and formal verification tools
- System identification and parameter estimation
  - New measurement techniques yield higher-quality data, but still noisy, sparse, heterogeneous
  - Large models on different time-scales, with many unobserved variables
- Systematic design of experimental perturbations for identification and control
- Redesign of networks for biotechnological or therapeutic purposes (synthetic biology)

Internships in IBIS

- Challenging problems for biologists, physicists, computer scientists, mathematicians, …
- … in a multidisciplinary working environment.
- Contact: Hidde.deJong@inria.fr and www.ibis.fr

Courtesy Guillaume Baptist (2008)

Contributors and sponsors

Grégory Batt, INRIA Paris-Rocquencourt
Valentina Baldazzi, INRA Avignon
Bruno Besson, INRIA Grenoble-Rhône-Alpes
Eugenio Cinquemani, INRIA Grenoble-Rhône-Alpes
Hidde de Jong, INRIA Grenoble-Rhône-Alpes
Estelle Dumas, INRIA Grenoble-Rhône-Alpes
Johannes Geiselmann, Université Joseph Fourier, Grenoble
Jean-Luc Gouzé, INRIA Sophia-Antipolis-Méditerranée
Radu Mateescu, INRIA Grenoble-Rhône-Alpes
Pedro Monteiro, INRIA Grenoble-Rhône-Alpes/IST Lisbon
Michel Page, INRIA Grenoble-Rhône-Alpes/Université Pierre Mendès France, Grenoble
Corinne Pinel, Université Joseph Fourier, Grenoble
Caroline Ranquet, Université Joseph Fourier, Grenoble
Delphine Ropers, INRIA Grenoble-Rhône-Alpes

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